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(54) Title: PROTEIN VARIANTS HAVING MODIFIED IMMUNOGENICITY

(57) Abstract: The present invention relates to a method of selecting a protein variant having modified immunogenicity as compared to the parent protein comprising the steps obtaining antibody binding peptide sequences, using the sequences to localise epitope sequences on the 3-dimensional structure of parent protein, defining an epitope area including amino acids situated within 5 Å from the epitope amino acids constituting the epitope sequence, changing one or more of the amino acids defining the epitope area of the parent protein by genetical engineering mutations of a DNA sequence encoding the parent protein, introducing the mutated DNA sequence into a suitable host, culturing said host and expressing the protein variant, and evaluating the immunogenicity of the protein variant using the parent protein as reference. The invention further relates to the protein variant and use thereof, as well as to a method for producing said protein variant.

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PROTEIN VARIANTS HAVING MODIFIED IMMUNOGENICITY**Field of invention**

5 The present invention relates to a method of selecting a protein variant having modified immunogenicity as compared to the parent protein, to the protein variant and use thereof, as well as to a method for producing said protein variant.

10 Background of the invention

An increasing number of proteins, including enzymes, are being produced industrially, for use in various industries, housekeeping and medicine. Being proteins they are likely to stimulate an
15 immunological response in man and animals, including an allergic response.

Depending on the application, individuals get sensitised to the respective allergens by inhalation, direct contact with skin and
20 eyes, or injection. The general mechanism behind an allergic response is divided in a sensitisation phase and a symptomatic phase. The sensitisation phase involves a first exposure of an individual to an allergen. This event activates specific T- and B-lymphocytes, and leads to the production of allergen specific
25 IgE antibodies (in the present context the antibodies are denoted as usual, i.e. immunoglobulin E is IgE etc.). These IgE antibodies eventually facilitate allergen capturing and presentation to T-lymphocytes at the onset of the symptomatic phase. This phase is initiated by a second exposure to the same or a
30 resembling antigen. The specific IgE antibodies bind to the specific IgE receptors on mast cells and basophils, among others, and capture at the same time the allergen. The polyclonal nature of this process results in bridging and clustering of the IgE receptors, and subsequently in the activation of mast cells and

basophils. This activation triggers the release of various chemical mediators involved in the early as well as late phase reactions of the symptomatic phase of allergy. Prevention of allergy in susceptible individuals is therefore a research area of great importance.

For certain forms of IgE-mediated allergies, a therapy exists, which comprises repeated administration of allergen preparations called 'allergen vaccines' (Int. Arch. Allergy Immunol., 1999, vol. 119, pp1-5). This leads to reduction of the allergic symptoms, possibly due to a redirection of the immune response away from the allergic (Th2) pathway and towards the immunoprotective (Th1) pathway (Int. Arch. Allergy Immunol., 1999, vol. 119, pp1-5).

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Various attempts to reduce the immunogenicity of polypeptides and proteins have been conducted. It has been found that small changes in an epitope may affect the binding to an antibody. This may result in a reduced importance of such an epitope, maybe converting it from a high affinity to a low affinity epitope, or maybe even result in epitope loss, i.e. that the epitope cannot sufficiently bind an antibody to elicit an immunogenic response.

25 There is a need for methods to identify epitopes on proteins and alter these epitopes in order to modify the immunogenicity of proteins in a targeted manner. Such methods and kits for their execution can have at least four useful purposes:

- 30 1) reduce the allergenicity of a commercial protein using protein engineering.
- 2) reduce the potential of commercial proteins to cross-react with environmental allergens and hence cause allergic reactions

in people sensitized to the environmental allergens (or vice versa).

- 3) improve the immunotherapeutic effect of allergen vaccines.
- 4) assist characterization of clinical allergies in order to select the appropriate treatment, including allergen vaccination.

In WO99/53038 (Genencor Int.) as well as in prior references (Kammerer et al, Clin. Exp. Allergy, 1997, vol. 27, pp 1016-1026; Sakakibara et al, J. Vet. Med. Sci., 1998; vol. 60, pp. 599-605), methods are described, which identify linear T-cell epitopes among a library of known peptide sequences, each representing part of the primary sequence of the protein of interest. Further, several similar techniques for localization of B-cell epitopes are disclosed by Walsh et al, J. Immunol. Methods, vol. 121, 1275-280, (1989), and by Schoofs et al. J. Immunol. vol. 140, 611-616, (1987). All of these methods, however, only leads to identification of linear epitopes, not to identification of 'structural' or 'discontinuous' epitopes, which are found on the 3-dimensional surface of protein molecules and which comprise amino acids from several discrete sites of the primary sequence of the protein. For several allergens, it has been realized that the dominant epitopes are of such discontinuous nature (Collins et al., Clin. Exp. All. 1996, vol. 26, pp. 36-42).

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Slootstra et al; Molecular Diversity, 2, pp. 156-164, 1996 disclose the screening of a semi-random library of synthetic peptides for their binding properties to three monoclonal antibodies by immobilizing the peptides on polyethylene pins and binding a dilution series of each antibody to the pins. This reference does not disclose any indication of how the antibody binding peptide sequences relate to any full protein antigens or allergens.

In WO92/10755 a method for modifying proteins to obtain less immunogenic variants is described. Randomly constructed protein variants, revealing a reduced binding of antibodies to the parent enzyme as compared to the parent enzyme itself, are selected for the measurement in animal models in terms of allergenicity. Finally, it is assessed whether reduction in immunogenicity is due to true elimination of an epitope or a reduction in affinity for antibodies. This method targets the identification of amino acids that may be part of structural epitopes by using a complete protein for assessing antigen binding. The major drawbacks of this approach are the 'trial and error' character, which makes it a lengthy and expensive process, and the lack of general information on the epitope patterns. Without this information, the results obtained for one protein can not be applied on another protein.

WO 99/47680 (ALK-ABELLÓ) discloses the identification and modification of B-cell epitopes by protein engineering. However, the method is based on crystal structures of Fab-antigen complexes, and B-cell epitopes are defined as "a section of the surface of the antigen comprising 15-25 amino acid residues, which are within a distance from the atoms of the antibody enabling direct interaction" (p.3). This publication does not show how one selects which Fab fragment to use (e.g. to target the most dominant allergy epitopes) or how one selects the substitutions to be made. Further, their method cannot be used in the absence of such crystallographic data for antigen-antibody complexes, which are very cumbersome, sometimes impossible, to obtain - especially since one would need a separate crystal structure for each epitope to be changed.

Hence, it is of interest to establish a general and efficient method to identify structural epitopes on the 3-dimensional surface of commercial and environmental allergens.

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Summary of the invention

The present invention relates to a method of selecting a protein variant having modified immunogenicity as compared to a parent
10 protein,

comprising the steps of:

a) obtaining antibody binding peptide sequences,
15

b) using the sequences to localise epitope sequences on the 3-dimensional structure of parent protein,

c) defining an epitope area including amino acids situated
20 within 5 Å from the epitope amino acids constituting the epitope sequence,

d) changing one or more of the amino acids defining the epitope area of the parent protein by genetic engineering mutations of a
25 DNA sequence encoding the parent protein,

e) introducing the mutated DNA sequence into a suitable host, culturing said host and expressing the protein variant, and

30 f) evaluating the immunogenicity of the protein variant using the parent protein as reference.

A second aspect of the present invention is a protein variant having modified immunogenicity as compared to its parent protein. The amino acid sequence of the protein variant differs from the amino acid sequence of the parent protein with respect
5 to at least one epitope pattern of the parent protein, such that the immunogenicity of the protein variant is modified as compared with the immunogenicity of the parent protein.

A further aspect of the present invention is a composition comprising a protein variant as defined above, as well as the use
10 of the composition for industrial application, such as the production of a formulation for personal care products (for example shampoo; soap; skin, hand and face lotions; skin, hand and face crèmes; hair dyes; toothpaste), food (for example in the baking
15 industry), detergents and for the production of pharmaceuticals, e.g. vaccines.

Yet another aspect is a DNA molecule encoding a protein variant as defined above.

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Further aspects are a vector comprising a DNA molecule as described above as well a host cell comprising said DNA molecule.

Another aspect is a method of producing a protein variant having
25 modified immunogenicity as compared to the parent protein as defined above.

Definitions

30

Prior to a discussion of the detailed embodiments of the invention, a definition of specific terms related to the main aspects of the invention is provided.

In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, Fritsch
5 & Maniatis, *Molecular Cloning: A Laboratory Manual*, Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (herein "Sambrook et al., 1989"); *DNA Cloning: A Practical Approach*, Volumes I and II /D.N. Glover ed. 1985); *Oligonucleotide Synthesis* (M.J. Gait ed. 1984); *Nucleic Acid Hy-*
10 *bridization* (B.D. Hames & S.J. Higgins eds (1985)); *Transcription And Translation* (B.D. Hames & S.J. Higgins, eds. (1984)); *Animal Cell Culture* (R.I. Freshney, ed. (1986)); *Immobilized Cells And Enzymes* (IRL Press, (1986)); B. Perbal, *A Practical Guide To Molecular Cloning* (1984).

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When applied to a protein, the term "isolated" indicates that the protein is found in a condition other than its native environment, such as apart from blood and animal tissue. In a preferred form, the isolated protein is substantially free of other
20 proteins, particularly other proteins of animal origin. It is preferred to provide the proteins in a highly purified form, i.e., greater than 95% pure, more preferably greater than 99% pure. When applied to a polynucleotide molecule, the term "isolated" indicates that the molecule is removed from its natural
25 genetic milieu, and is thus free of other extraneous or unwanted coding sequences, and is in a form suitable for use within genetically engineered protein production systems. Such isolated molecules are those that are separated from their natural environment and include cDNA and genomic clones. Isolated DNA mole-
30 cules of the present invention are free of other genes with which they are ordinarily associated, and may include naturally occurring 5' and 3' untranslated regions such as promoters and terminators. The identification of associated regions will be

evident to one of ordinary skill in the art (see for example, Dynan and Tijan, Nature 316: 774-78, 1985).

A "polynucleotide" is a single- or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. Polynucleotides include RNA and DNA, and may be isolated from natural sources, synthesized in vitro, or prepared from a combination of natural and synthetic molecules.

10 A "nucleic acid molecule" refers to the phosphate ester polymeric form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules") in either single stranded form, or a double-
15 stranded helix. Double stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible. The term nucleic acid molecule, and in particular DNA or RNA molecule, refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary or quaternary forms. Thus, this term in-
20 cludes double-stranded DNA found, inter alia, in linear or circular DNA molecules (e.g., restriction fragments), plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in
25 the 5' to 3' direction along the nontranscribed strand of DNA (i.e., the strand having a sequence homologous to the mRNA). A "recombinant DNA molecule" is a DNA molecule that has undergone a molecular biological manipulation.

30 A DNA "coding sequence" is a double-stranded DNA sequence, which is transcribed and translated into a polypeptide in a cell in vitro or in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a

translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. If the coding sequence is intended for expression in a eukaryotic cell, a polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

10 An "Expression vector" is a DNA molecule, linear or circular, that comprises a segment encoding a polypeptide of interest operably linked to additional segments that provide for its transcription. Such additional segments may include promoter and terminator sequences, and optionally one or more origins of replication, one or more selectable markers, an enhancer, a polyadenylation signal, and the like. Expression vectors are generally derived from plasmid or viral DNA, or may contain elements of both.

20 Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, terminators, and the like, that provide for the expression of a coding sequence in a host cell. In eukaryotic cells, polyadenylation signals are control sequences.

25

A "secretory signal sequence" is a DNA sequence that encodes a polypeptide (a "secretory peptide" that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in which it is synthesized. The larger polypeptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

30

The term "promoter" is used herein for its art-recognized meaning to denote a portion of a gene containing DNA sequences that

provide for the binding of RNA polymerase and initiation of transcription. Promoter sequences are commonly, but not always, found in the 5' non-coding regions of genes.

5 "Operably linked", when referring to DNA segments, indicates that the segments are arranged so that they function in concert for their intended purposes, e.g. transcription initiates in the promoter and proceeds through the coding segment to the terminator.

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A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then trans-
RNA spliced and translated into the protein encoded by the cod-
15 ing sequence.

"Isolated polypeptide" is a polypeptide which is essentially free of other non-[enzyme] polypeptides, e.g., at least about 20% pure, preferably at least about 40% pure, more preferably
20 about 60% pure, even more preferably about 80% pure, most preferably about 90% pure, and even most preferably about 95% pure, as determined by SDS-PAGE.

"Heterologous" DNA refers to DNA not naturally located in the
25 cell, or in a chromosomal site of the cell. Preferably, the heterologous DNA includes a gene foreign to the cell.

A cell has been "transfected" by exogenous or heterologous DNA when such DNA has been introduced inside the cell. A cell has
30 been "transformed" by exogenous or heterologous DNA when the transfected DNA effects a phenotypic change. Preferably, the transforming DNA should be integrated (covalently linked) into chromosomal DNA making up the genome of the cell.

A "clone" is a population of cells derived from a single cell or common ancestor by mitosis.

"Homologous recombination" refers to the insertion of a foreign DNA sequence of a vector in a chromosome. Preferably, the vector targets a specific chromosomal site for homologous recombination. For specific homologous recombination, the vector will contain sufficiently long regions of homology to sequences of the chromosome to allow complementary binding and incorporation of the vector into the chromosome. Longer regions of homology, and greater degrees of sequence similarity, may increase the efficiency of homologous recombination.

Nucleic Acid Sequence

The techniques used to isolate or clone a nucleic acid sequence encoding a polypeptide are known in the art and include isolation from genomic DNA, preparation from cDNA, or a combination thereof. The cloning of the nucleic acid sequences of the present invention from such genomic DNA can be effected, e.g., by using the well known polymerase chain reaction (PCR) or antibody screening of expression libraries to detect cloned DNA fragments with shared structural features. See, e.g., Innis et al., 1990, A Guide to Methods and Application, Academic Press, New York. Other nucleic acid amplification procedures such as ligase chain reaction (LCR), ligated activated transcription (LAT) and nucleic acid sequence-based amplification (NASBA) may be used. The nucleic acid sequence may be cloned from a strain producing the polypeptide, or from another related organism and thus, for example, may be an allelic or species variant of the polypeptide encoding region of the nucleic acid sequence.

The term "isolated" nucleic acid sequence as used herein refers to a nucleic acid sequence which is essentially free of other nucleic acid sequences, e.g., at least about 20% pure, prefera-

bly at least about 40% pure, more preferably about 60% pure, even more preferably about 80% pure, most preferably about 90% pure, and even most preferably about 95% pure, as determined by agarose gel electrophoresis. For example, an isolated nucleic acid sequence can be obtained by standard cloning procedures used in genetic engineering to relocate the nucleic acid sequence from its natural location to a different site where it will be reproduced. The cloning procedures may involve excision and isolation of a desired nucleic acid fragment comprising the nucleic acid sequence encoding the polypeptide, insertion of the fragment into a vector molecule, and incorporation of the recombinant vector into a host cell where multiple copies or clones of the nucleic acid sequence will be replicated. The nucleic acid sequence may be of genomic, cDNA, RNA, semisynthetic, synthetic origin, or any combinations thereof.

Nucleic Acid Construct

As used herein the term "nucleic acid construct" is intended to indicate any nucleic acid molecule of cDNA, genomic DNA, synthetic DNA or RNA origin. The term "construct" is intended to indicate a nucleic acid segment which may be single- or double-stranded, and which may be based on a complete or partial naturally occurring nucleotide sequence encoding a polypeptide of interest. The construct may optionally contain other nucleic acid segments.

The DNA of interest may suitably be of genomic or cDNA origin, for instance obtained by preparing a genomic or cDNA library and screening for DNA sequences coding for all or part of the polypeptide by hybridization using synthetic oligonucleotide probes in accordance with standard techniques (cf. Sambrook et al., *supra*).

The nucleic acid construct may also be prepared synthetically by established standard methods, e.g. the phosphoamidite method described by Beaucage and Caruthers, Tetrahedron Letters 22 (1981), 1859 - 1869, or the method described by Matthes et al.,
5 EMBO Journal 3 (1984), 801 - 805. According to the phosphoamidite method, oligonucleotides are synthesized, e.g. in an automatic DNA synthesizer, purified, annealed, ligated and cloned in suitable vectors.

10 Furthermore, the nucleic acid construct may be of mixed synthetic and genomic, mixed synthetic and cDNA or mixed genomic and cDNA origin prepared by ligating fragments of synthetic, genomic or cDNA origin (as appropriate), the fragments corresponding to various parts of the entire nucleic acid construct, in
15 accordance with standard techniques.

The nucleic acid construct may also be prepared by polymerase chain reaction using specific primers, for instance as described in US 4,683,202 or Saiki et al., Science 239 (1988), 487 - 491.

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The term nucleic acid construct may be synonymous with the term expression cassette when the nucleic acid construct contains all the control sequences required for expression of a coding sequence of the present invention. The term "coding sequence" as
25 defined herein is a sequence which is transcribed into mRNA and translated into a polypeptide of the present invention when placed under the control of the above mentioned control sequences. The boundaries of the coding sequence are generally determined by a translation start codon ATG at the 5'-terminus
30 and a translation stop codon at the 3'-terminus. A coding sequence can include, but is not limited to, DNA, cDNA, and recombinant nucleic acid sequences.

The term "control sequences" is defined herein to include all components which are necessary or advantageous for expression of the coding sequence of the nucleic acid sequence. Each control sequence may be native or foreign to the nucleic acid sequence
5 encoding the polypeptide. Such control sequences include, but are not limited to, a leader, a polyadenylation sequence, a propeptide sequence, a promoter, a signal sequence, and a transcription terminator. At a minimum, the control sequences include a promoter, and transcriptional and translational stop
10 signals. The control sequences may be provided with linkers for the purpose of introducing specific restriction sites facilitating ligation of the control sequences with the coding region of the nucleic acid sequence encoding a polypeptide.

15 The control sequence may be an appropriate promoter sequence, a nucleic acid sequence which is recognized by a host cell for expression of the nucleic acid sequence. The promoter sequence contains transcription and translation control sequences which mediate the expression of the polypeptide. The promoter may be
20 any nucleic acid sequence which shows transcriptional activity in the host cell of choice and may be obtained from genes encoding extracellular or intracellular polypeptides either homologous or heterologous to the host cell.

The control sequence may also be a suitable transcription terminator sequence, a sequence recognized by a host cell to terminate transcription. The terminator sequence is operably linked to the 3' terminus of the nucleic acid sequence encoding the polypeptide. Any terminator which is functional in the host cell of choice may be used
25
30 in the present invention.

The control sequence may also be a polyadenylation sequence, a sequence which is operably linked to the 3' terminus of the nucleic acid sequence and which, when transcribed, is recognized

by the host cell as a signal to add polyadenosine residues to transcribed mRNA. Any polyadenylation sequence which is functional in the host cell of choice may be used in the present invention.

5

The control sequence may also be a signal peptide coding region, which codes for an amino acid sequence linked to the amino terminus of the polypeptide which can direct the expressed polypeptide into the cell's secretory pathway of the host cell. The 5' end of the coding sequence of the nucleic acid sequence may inherently contain a signal peptide coding region naturally linked in translation reading frame with the segment of the coding region which encodes the secreted polypeptide. Alternatively, the 5' end of the coding sequence may contain a signal peptide coding region which is foreign to that portion of the coding sequence which encodes the secreted polypeptide. A foreign signal peptide coding region may be required where the coding sequence does not normally contain a signal peptide coding region. Alternatively, the foreign signal peptide coding region may simply replace the natural signal peptide coding region in order to obtain enhanced secretion relative to the natural signal peptide coding region normally associated with the coding sequence. The signal peptide coding region may be obtained from a glucoamylase or an amylase gene from an *Aspergillus* species, a lipase or protease gene from a *Rhizomucor* species, the gene for the alpha-factor from *Saccharomyces cerevisiae*, an amylase or a protease gene from a *Bacillus* species, or the calf preprochymosin gene. However, any signal peptide coding region capable of directing the expressed polypeptide into the secretory pathway of a host cell of choice may be used in the present invention.

The control sequence may also be a propeptide coding region, which codes for an amino acid sequence positioned at the amino terminus of a polypeptide. The resultant polypeptide is known as a proenzyme or propolypeptide (or a zymogen in some cases).

A propolypeptide is generally inactive and can be converted to mature active polypeptide by catalytic or autocatalytic cleavage of the propeptide from the propolypeptide. The propeptide coding region may be obtained from the *Bacillus subtilis* alkaline protease gene (aprE), the *Bacillus subtilis* neutral protease gene (nprT), the *Saccharomyces cerevisiae* alpha-factor gene, or the *Myceliophthora thermophilum* laccase gene (WO 95/33836).

The nucleic acid constructs of the present invention may also comprise one or more nucleic acid sequences which encode one or more factors that are advantageous in the expression of the polypeptide, e.g., an activator (e.g., a trans-acting factor), a chaperone, and a processing protease. Any factor that is functional in the host cell of choice may be used in the present invention. The nucleic acids encoding one or more of these factors are not necessarily in tandem with the nucleic acid sequence encoding the polypeptide.

An activator is a protein which activates transcription of a nucleic acid sequence encoding a polypeptide (Kudla et al., 1990, EMBO Journal 9:1355-1364; Jarai and Buxton, 1994, Current Genetics 26:2238-244; Verdier, 1990, Yeast 6:271-297). The nucleic acid sequence encoding an activator may be obtained from the genes encoding *Bacillus stearothermophilus* NprA (nprA), *Saccharomyces cerevisiae* heme activator protein 1 (hap1), *Saccharomyces cerevisiae* galactose metabolizing protein 4 (gal4), and *Aspergillus nidulans* ammonia regulation protein (areA). For further examples, see Verdier, 1990, supra and MacKenzie et al., 1993, Journal of General Microbiology 139:2295-2307.

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A chaperone is a protein which assists another polypeptide in folding properly (Hartl et al., 1994, TIBS 19:20-25; Bergeron et al., 1994, TIBS 19:124-128; Demolder et al., 1994, Journal of Biotechnology 32:179-189; Craig, 1993, Science 260:1902-1903;

Gething and Sambrook, 1992, *Nature* 355:33-45; Puig and Gilbert, 1994, *Journal of Biological Chemistry* 269:7764-7771; Wang and Tsou, 1993, *The FASEB Journal* 7:1515-11157; Robinson et al., 1994, *Bio/Technology* 1:381-384). The nucleic acid sequence encoding a chaperone may be obtained from the genes encoding *Bacillus subtilis* GroE proteins, *Aspergillus oryzae* protein disulphide isomerase, *Saccharomyces cerevisiae* calnexin, *Saccharomyces cerevisiae* BiP/GRP78, and *Saccharomyces cerevisiae* Hsp70. For further examples, see Gething and Sambrook, 1992, *supra*, and
10 Hartl et al., 1994, *supra*.

A processing protease is a protease that cleaves a propeptide to generate a mature biochemically active polypeptide (Enderlin and Ogrydziak, 1994, *Yeast* 10:67-79; Fuller et al., 1989, *Proceedings of the National Academy of Sciences USA* 86:1434-1438; Julius et al., 1984, *Cell* 37:1075-1089; Julius et al., 1983, *Cell* 32:839-852). The nucleic acid sequence encoding a processing protease may be obtained from the genes encoding *Aspergillus niger* Kex2, *Saccharomyces cerevisiae* dipeptidylaminopeptidase, 20 *Saccharomyces cerevisiae* Kex2, and *Yarrowia lipolytica* dibasic processing endoprotease (xpr6).

It may also be desirable to add regulatory sequences which allow the regulation of the expression of the polypeptide relative to
25 the growth of the host cell. Examples of regulatory systems are those which cause the expression of the gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Regulatory systems in prokaryotic systems would include the lac, tac, and trp operator
30 systems. In yeast, the ADH2 system or GAL1 system may be used. In filamentous fungi, the TAKA alpha-amylase promoter, *Aspergillus niger* glucoamylase promoter, and the *Aspergillus oryzae* glucoamylase promoter may be used as regulatory sequences. Other examples of regulatory sequences are those which allow for gene

amplification. In eukaryotic systems, these include the dihydrofolate reductase gene which is amplified in the presence of methotrexate, and the metallothionein genes which are amplified with heavy metals. In these cases, the nucleic acid sequence encoding the polypeptide would be placed in tandem with the regulatory sequence.

Promoters

Examples of suitable promoters for directing the transcription of the nucleic acid constructs of the present invention, especially in a bacterial host cell, are the promoters obtained from the *E. coli* lac operon, the *Streptomyces coelicolor* agarase gene (*dagA*), the *Bacillus subtilis* levansucrase gene (*sacB*), the *Bacillus subtilis* alkaline protease gene, the *Bacillus licheniformis* alpha-amylase gene (*amyL*), the *Bacillus stearothermophilus* maltogenic amylase gene (*amyM*), the *Bacillus amyloliquefaciens* alpha-amylase gene (*amyQ*), the *Bacillus amyloliquefaciens* BAN amylase gene, the *Bacillus licheniformis* penicillinase gene (*penP*), the *Bacillus subtilis* *xylA* and *xylB* genes, and the prokaryotic beta-lactamase gene (Villa-Kamaroff et al., 1978, Proceedings of the National Academy of Sciences USA 75:3727-3731), as well as the *tac* promoter (DeBoer et al., 1983, Proceedings of the National Academy of Sciences USA 80:21-25), or the *Bacillus pumilus* xylosidase gene, or by the phage Lambda PR or PL promoters or the *E. coli* lac, *trp* or *tac* promoters. Further promoters are described in "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94; and in Sambrook et al., 1989, supra.

Examples of suitable promoters for directing the transcription of the nucleic acid constructs of the present invention in a filamentous fungal host cell are promoters obtained from the genes encoding *Aspergillus oryzae* TAKA amylase, *Rhizomucor miehei* aspartic proteinase, *Aspergillus niger* neutral al-

pha-amylase, *Aspergillus niger* acid stable alpha-amylase, *Aspergillus niger* or *Aspergillus awamori* glucoamylase (glaA), *Rhizomucor miehei* lipase, *Aspergillus oryzae* alkaline protease, *Aspergillus oryzae* triose phosphate isomerase, *Aspergillus nidulans* acetamidase, *Fusarium oxysporum* trypsin-like protease (as
5 described in U.S. Patent No. 4,288,627, which is incorporated herein by reference), and hybrids thereof. Particularly preferred promoters for use in filamentous fungal host cells are the TAKA amylase, NA2-tpi (a hybrid of the promoters from the
10 genes encoding *Aspergillus niger* neutral (-amylase and *Aspergillus oryzae* triose phosphate isomerase), and glaA promoters. Further suitable promoters for use in filamentous fungus host cells are the ADH3 promoter (McKnight et al., The EMBO J. 4 (1985), 2093 - 2099) or the tpiA promoter.

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Examples of suitable promoters for use in yeast host cells include promoters from yeast glycolytic genes (Hitzeman et al., J. Biol. Chem. 255 (1980), 12073 - 12080; Alber and Kawasaki, J. Mol. Appl. Gen. 1 (1982), 419 - 434) or alcohol dehydrogenase
20 genes (Young et al., in Genetic Engineering of Microorganisms for Chemicals (Hollaender et al, eds.), Plenum Press, New York, 1982), or the TPI1 (US 4,599,311) or ADH2-4c (Russell et al., Nature 304 (1983), 652 - 654) promoters.

25 Further useful promoters are obtained from the *Saccharomyces cerevisiae* enolase (ENO-1) gene, the *Saccharomyces cerevisiae* galactokinase gene (GAL1), the *Saccharomyces cerevisiae* alcohol dehydrogenase/glyceraldehyde-3-phosphate dehydrogenase genes (ADH2/GAP), and the *Saccharomyces cerevisiae* 3-phosphoglycerate
30 kinase gene. Other useful promoters for yeast host cells are described by Romanos et al., 1992, Yeast 8:423-488. In a mammalian host cell, useful promoters include viral promoters such as those from Simian Virus 40 (SV40), Rous sarcoma virus (RSV), adenovirus, and bovine papilloma virus (BPV).

Examples of suitable promoters for directing the transcription of the DNA encoding the polypeptide of the invention in mammalian cells are the SV40 promoter (Subramani et al., Mol. Cell Biol. 1 (1981), 854 -864), the MT-1 (metallothionein gene) promoter (Palmiter et al., Science 222 (1983), 809 - 814) or the adenovirus 2 major late promoter.

An example of a suitable promoter for use in insect cells is the polyhedrin promoter (US 4,745,051; Vasuvedan et al., FEBS Lett. 311, (1992) 7 - 11), the P10 promoter (J.M. Vlak et al., J. Gen. Virology 69, 1988, pp. 765-776), the Autographa californica polyhedrosis virus basic protein promoter (EP 397 485), the baculovirus immediate early gene 1 promoter (US 5,155,037; US 5,162,222), or the baculovirus 39K delayed-early gene promoter (US 5,155,037; US 5,162,222).

Terminators

Preferred terminators for filamentous fungal host cells are obtained from the genes encoding *Aspergillus oryzae* TAKA amylase, *Aspergillus niger* glucoamylase, *Aspergillus nidulans* anthranilate synthase, *Aspergillus niger* alpha-glucosidase, and *Fusarium oxysporum* trypsin-like protease. for fungal hosts) the TPI1 (Alber and Kawasaki, op. cit.) or ADH3 (McKnight et al., op. cit.) terminators.

Preferred terminators for yeast host cells are obtained from the genes encoding *Saccharomyces cerevisiae* enolase, *Saccharomyces cerevisiae* cytochrome C (CYC1), or *Saccharomyces cerevisiae* glyceraldehyde-3-phosphate dehydrogenase. Other useful terminators for yeast host cells are described by Romanos et al., 1992, supra.

Polyadenylation Signals

Preferred polyadenylation sequences for filamentous fungal host cells are obtained from the genes encoding *Aspergillus oryzae*

TAKA amylase, *Aspergillus niger* glucoamylase, *Aspergillus nidulans* anthranilate synthase, and *Aspergillus niger* alpha-glucosidase.

Useful polyadenylation sequences for yeast host cells are described by Guo and Sherman, 1995, *Molecular Cellular Biology* 15:5983-5990.

Polyadenylation sequences are well known in the art for mammalian host cells such as SV40 or the adenovirus 5 Elb region.

10 Signal Sequences

An effective signal peptide coding region for bacterial host cells is the signal peptide coding region obtained from the maltogenic amylase gene from *Bacillus* NCIB 11837, the *Bacillus stearothermophilus* alpha-amylase gene, the *Bacillus licheniformis* subtilisin gene, the *Bacillus licheniformis* beta-lactamase gene, the *Bacillus stearothermophilus* neutral proteases genes (nprT, nprS, nprM), and the *Bacillus subtilis* PrsA gene. Further signal peptides are described by Simonen and Palva, 1993, *Microbiological Reviews* 57:109-137.

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An effective signal peptide coding region for filamentous fungal host cells is the signal peptide coding region obtained from *Aspergillus oryzae* TAKA amylase gene, *Aspergillus niger* neutral amylase gene, the *Rhizomucor miehei* aspartic proteinase gene, the *Humicola lanuginosa* cellulase or lipase gene, or the *Rhizomucor miehei* lipase or protease gene, *Aspergillus* sp. amylase or glucoamylase, a gene encoding a *Rhizomucor miehei* lipase or protease. The signal peptide is preferably derived from a gene encoding *A. oryzae* TAKA amylase, *A. niger* neutral (-amylase, *A. niger* acid-stable amylase, or *A. niger* glucoamylase.

Useful signal peptides for yeast host cells are obtained from the genes for *Saccharomyces cerevisiae* a-factor and *Saccharomy-*

ces cerevisiae invertase. Other useful signal peptide coding regions are described by Romanos et al., 1992, supra.

For secretion from yeast cells, the secretory signal sequence may encode any signal peptide which ensures efficient direction of the expressed polypeptide into the secretory pathway of the cell. The signal peptide may be naturally occurring signal peptide, or a functional part thereof, or it may be a synthetic peptide. Suitable signal peptides have been found to be the a-factor signal peptide (cf. US 4,870,008), the signal peptide of mouse salivary amylase (cf. O. Hagenbuchle et al., Nature 289, 1981, pp. 643-646), a modified carboxypeptidase signal peptide (cf. L.A. Valls et al., Cell 48, 1987, pp. 887-897), the yeast BAR1 signal peptide (cf. WO 87/02670), or the yeast aspartic protease 3 (YAP3) signal peptide (cf. M. Egel-Mitani et al., Yeast 6, 1990, pp. 127-137).

For efficient secretion in yeast, a sequence encoding a leader peptide may also be inserted downstream of the signal sequence and upstream of the DNA sequence encoding the polypeptide. The function of the leader peptide is to allow the expressed polypeptide to be directed from the endoplasmic reticulum to the Golgi apparatus and further to a secretory vesicle for secretion into the culture medium (i.e. exportation of the polypeptide across the cell wall or at least through the cellular membrane into the periplasmic space of the yeast cell). The leader peptide may be the yeast a-factor leader (the use of which is described in e.g. US 4,546,082, EP 16 201, EP 123 294, EP 123 544 and EP 163 529). Alternatively, the leader peptide may be a synthetic leader peptide, which is to say a leader peptide not found in nature. Synthetic leader peptides may, for instance, be constructed as described in WO 89/02463 or WO 92/11378.

For use in insect cells, the signal peptide may conveniently be derived from an insect gene (cf. WO 90/05783), such as the lepidopteran *Manduca sexta* adipokinetic hormone precursor signal peptide (cf. US 5,023,328).

Expression Vectors

The present invention also relates to recombinant expression
5 vectors comprising a nucleic acid sequence of the present inven-
tion, a promoter, and transcriptional and translational stop
signals. The various nucleic acid and control sequences de-
scribed above may be joined together to produce a recombinant
expression vector which may include one or more convenient re-
10 striction sites to allow for insertion or substitution of the
nucleic acid sequence encoding the polypeptide at such sites.
Alternatively, the nucleic acid sequence of the present inven-
tion may be expressed by inserting the nucleic acid sequence or
a nucleic acid construct comprising the sequence into an appro-
15 priate vector for expression. In creating the expression vec-
tor, the coding sequence is located in the vector so that the
coding sequence is operably linked with the appropriate control
sequences for expression, and possibly secretion.

20 The recombinant expression vector may be any vector (e.g., a
plasmid or virus) which can be conveniently subjected to recom-
binant DNA procedures and can bring about the expression of the
nucleic acid sequence. The choice of the vector will typically
depend on the compatibility of the vector with the host cell
25 into which the vector is to be introduced. The vectors may be
linear or closed circular plasmids. The vector may be an
autonomously replicating vector, i.e., a vector which exists as
an extrachromosomal entity, the replication of which is inde-
pendent of chromosomal replication, e.g., a plasmid, an ex-
30 trachromosomal element, a minichromosome, or an artificial chro-
mosome. The vector may contain any means for assuring self-
replication. Alternatively, the vector may be one which, when
introduced into the host cell, is integrated into the genome and
replicated together with the chromosome(s) into which it has

been integrated. The vector system may be a single vector or plasmid or two or more vectors or plasmids which together contain the total DNA to be introduced into the genome of the host cell, or a transposon.

5

The vectors of the present invention preferably contain one or more selectable markers which permit easy selection of transformed cells. A selectable marker is a gene the product of which provides for biocide or viral resistance, resistance to heavy metals, prototrophy to auxotrophs, and the like. Examples of bacterial selectable markers are the *dal* genes from *Bacillus subtilis* or *Bacillus licheniformis*, or markers which confer antibiotic resistance such as ampicillin, kanamycin, chloramphenicol, tetracycline, neomycin, hygromycin or methotrexate resistance. A frequently used mammalian marker is the dihydrofolate reductase gene (DHFR). Suitable markers for yeast host cells are ADE2, HIS3, LEU2, LYS2, MET3, TRP1, and URA3. A selectable marker for use in a filamentous fungal host cell may be selected from the group including, but not limited to, *amdS* (acetamidase), *argB* (ornithine carbamoyltransferase), *bar* (phosphinothricin acetyltransferase), *hygB* (hygromycin phosphotransferase), *niaD* (nitrate reductase), *pyrG* (orotidine-5'-phosphate decarboxylase), *sC* (sulfate adenylyltransferase), *trpC* (anthranilate synthase), and glufosinate resistance markers, as well as equivalents from other species. Preferred for use in an *Aspergillus* cell are the *amdS* and *pyrG* markers of *Aspergillus nidulans* or *Aspergillus oryzae* and the *bar* marker of *Streptomyces hygroscopicus*. Furthermore, selection may be accomplished by co-transformation, e.g., as described in WO 91/17243, where the selectable marker is on a separate vector.

The vectors of the present invention preferably contain an element(s) that permits stable integration of the vector into the

host cell genome or autonomous replication of the vector in the cell independent of the genome of the cell.

The vectors of the present invention may be integrated into the host cell genome when introduced into a host cell. For integration, the vector may rely on the nucleic acid sequence encoding the polypeptide or any other element of the vector for stable integration of the vector into the genome by homologous or non-homologous recombination. Alternatively, the vector may contain additional nucleic acid sequences for directing integration by homologous recombination into the genome of the host cell. The additional nucleic acid sequences enable the vector to be integrated into the host cell genome at a precise location(s) in the chromosome(s). To increase the likelihood of integration at a precise location, the integrational elements should preferably contain a sufficient number of nucleic acids, such as 100 to 1,500 base pairs, preferably 400 to 1,500 base pairs, and most preferably 800 to 1,500 base pairs, which are highly homologous with the corresponding target sequence to enhance the probability of homologous recombination. The integrational elements may be any sequence that is homologous with the target sequence in the genome of the host cell. Furthermore, the integrational elements may be non-encoding or encoding nucleic acid sequences. On the other hand, the vector may be integrated into the genome of the host cell by non-homologous recombination. These nucleic acid sequences may be any sequence that is homologous with a target sequence in the genome of the host cell, and, furthermore, may be non-encoding or encoding sequences.

For autonomous replication, the vector may further comprise an origin of replication enabling the vector to replicate autonomously in the host cell in question. Examples of bacterial origins of replication are the origins of replication of plasmids pBR322, pUC19, pACYC177, pACYC184, pUB110, pE194, pTA1060, and

pAMS1. Examples of origin of replications for use in a yeast host cell are the 2 micron origin of replication, the combination of CEN6 and ARS4, and the combination of CEN3 and ARS1. The origin of replication may be one having a mutation which makes its functioning temperature-sensitive in the host cell (see, e.g., Ehrlich, 1978, Proceedings of the National Academy of Sciences USA 75:1433).

More than one copy of a nucleic acid sequence encoding a polypeptide of the present invention may be inserted into the host cell to amplify expression of the nucleic acid sequence. Stable amplification of the nucleic acid sequence can be obtained by integrating at least one additional copy of the sequence into the host cell genome using methods well known in the art and selecting for transformants.

The procedures used to ligate the elements described above to construct the recombinant expression vectors of the present invention are well known to one skilled in the art (see, e.g., Sambrook et al., 1989, supra).

20

Host Cells

The present invention also relates to recombinant host cells, comprising a nucleic acid sequence of the invention, which are advantageously used in the recombinant production of the polypeptides. The term "host cell" encompasses any progeny of a parent cell which is not identical to the parent cell due to mutations that occur during replication.

The cell is preferably transformed with a vector comprising a nucleic acid sequence of the invention followed by integration of the vector into the host chromosome. "Transformation" means introducing a vector comprising a nucleic acid sequence of the present invention into a host cell so that the vector is maintained as a chromosomal integrant or as a self-replicating ex-

tra-chromosomal vector. Integration is generally considered to be an advantage as the nucleic acid sequence is more likely to be stably maintained in the cell. Integration of the vector into the host chromosome may occur by homologous or non-homologous recombination as described above.

The choice of a host cell will to a large extent depend upon the gene encoding the polypeptide and its source. The host cell may be a unicellular microorganism, e.g., a prokaryote, or a non-unicellular microorganism, e.g., a eukaryote. Useful unicellular cells are bacterial cells such as gram positive bacteria including, but not limited to, a *Bacillus* cell, e.g., *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus circulans*, *Bacillus coagulans*, *Bacillus lautus*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus stearothermophilus*, *Bacillus subtilis*, and *Bacillus thuringiensis*; or a *Streptomyces* cell, e.g., *Streptomyces lividans* or *Streptomyces murinus*, or gram negative bacteria such as *E. coli* and *Pseudomonas* sp. In a preferred embodiment, the bacterial host cell is a *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus stearothermophilus* or *Bacillus subtilis* cell. The transformation of a bacterial host cell may, for instance, be effected by protoplast transformation (see, e.g., Chang and Cohen, 1979, *Molecular General Genetics* 168:111-115), by using competent cells (see, e.g., Young and Spizizin, 1961, *Journal of Bacteriology* 81:823-829, or Dubnar and Davidoff-Abelson, 1971, *Journal of Molecular Biology* 56:209-221), by electroporation (see, e.g., Shigekawa and Dower, 1988, *Biotechniques* 6:742-751), or by conjugation (see, e.g., Koehler and Thorne, 1987, *Journal of Bacteriology* 169:5771-5278).

The host cell may be a eukaryote, such as a mammalian cell, an insect cell, a plant cell or a fungal cell.

Useful mammalian cells include Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, COS cells, or any number of other immortalized cell lines available, e.g., from the American Type Culture Collection.

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Examples of suitable mammalian cell lines are the COS (ATCC CRL 1650 and 1651), BHK (ATCC CRL 1632, 10314 and 1573, ATCC CCL 10), CHL (ATCC CCL39) or CHO (ATCC CCL 61) cell lines. Methods of transfecting mammalian cells and expressing DNA sequences introduced in the cells are described in e.g. Kaufman and Sharp, J. Mol. Biol. 159 (1982), 601 - 621; Southern and Berg, J. Mol. Appl. Genet. 1 (1982), 327 - 341; Loyter et al., Proc. Natl. Acad. Sci. USA 79 (1982), 422 - 426; Wigler et al., Cell 14 (1978), 725; Corsaro and Pearson, Somatic Cell Genetics 7 (1981), 603; Ausubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, Inc., N.Y., 1987, Hawley-Nelson et al., Focus 15 (1993), 73; Ciccarone et al., Focus 15 (1993), 80; Graham and van der Eb, Virology 52 (1973), 456; and Neumann et al., EMBO J. 1 (1982), 841 - 845.

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In a preferred embodiment, the host cell is a fungal cell. "Fungi" as used herein includes the phyla Ascomycota, Basidiomycota, Chytridiomycota, and Zygomycota (as defined by Hawksworth et al., In, Ainsworth and Bisby's Dictionary of The Fungi, 8th edition, 1995, CAB International, University Press, Cambridge, UK) as well as the Oomycota (as cited in Hawksworth et al., 1995, supra, page 171) and all mitosporic fungi (Hawksworth et al., 1995, supra). Representative groups of Ascomycota include, e.g., Neurospora, Eupenicillium (=Penicillium), Emericella (=Aspergillus), Eurotium (=Aspergillus), and the true yeasts listed above. Examples of Basidiomycota include mushrooms, rusts, and smuts. Representative groups of Chytridiomycota include, e.g., Allomyces, Blastocladiella, Coelomomyces, and aquatic fungi. Representative groups of Oomycota include, e.g.,

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Saprolegniomycetous aquatic fungi (water molds) such as Achlya. Examples of mitosporic fungi include Aspergillus, Penicillium, Candida, and Alternaria. Representative groups of Zygomycota include, e.g., Rhizopus and Mucor.

5 In a preferred embodiment, the fungal host cell is a yeast cell. "Yeast" as used herein includes ascosporogenous yeast (Endomycetales), basidiosporogenous yeast, and yeast belonging to the Fungi Imperfecti (Blastomycetes). The ascosporogenous yeasts are divided into the families Spermophthoraceae and Saccharomycetaceae. The latter is comprised of four subfamilies, 10 Schizosaccharomycoideae (e.g., genus Schizosaccharomyces), Nadsonioideae, Lipomycoideae, and Saccharomycoideae (e.g., genera Pichia, Kluyveromyces and Saccharomyces). The basidiosporogenous yeasts include the genera Leucosporidium, Rhodosporidium, 15 Sporidiobolus, Filobasidium, and Filobasidiella. Yeast belonging to the Fungi Imperfecti are divided into two families, Sporobolomycetaceae (e.g., genera Sporobolomyces and Bullera) and Cryptococcaceae (e.g., genus Candida). Since the classification of yeast may change in the future, for the purposes of this invention, yeast shall be defined as described in Biology and Activities of Yeast (Skinner, F.A., Passmore, S.M., and Davenport, R.R., eds, Soc. App. Bacteriol. Symposium Series No. 9, 1980. The biology of yeast and manipulation of yeast genetics are well known in the art (see, e.g., Biochemistry and Genetics of Yeast, 25 Bacil, M., Horecker, B.J., and Stopani, A.O.M., editors, 2nd edition, 1987; The Yeasts, Rose, A.H., and Harrison, J.S., editors, 2nd edition, 1987; and The Molecular Biology of the Yeast Saccharomyces, Strathern et al., editors, 1981).

30 The yeast host cell may be selected from a cell of a species of Candida, Kluyveromyces, Saccharomyces, Schizosaccharomyces, Candida, Pichia, Hansenula, , or Yarrowia. In a preferred embodiment, the yeast host cell is a Saccharomyces carlsbergensis, Saccharomyces cerevisiae, Saccharomyces diastaticus, Saccharomy-

ces douglasii, Saccharomyces kluyveri, Saccharomyces norbensis or Saccharomyces oviformis cell. Other useful yeast host cells are a Kluyveromyces lactis Kluyveromyces fragilis Hansenula polymorpha, Pichia pastoris Yarrowia lipolytica, Schizosaccharomyces pombe, Ustilgo maylis, Candida maltose, Pichia guilliermondii and Pichia methanolio cell (cf. Gleeson et al., J. Gen. Microbiol. 132, 1986, pp. 3459-3465; US 4,882,279 and US 4,879,231).

10 In a preferred embodiment, the fungal host cell is a filamentous fungal cell. "Filamentous fungi" include all filamentous forms of the subdivision Eumycota and Oomycota (as defined by Hawksworth et al., 1995, supra). The filamentous fungi are characterized by a vegetative mycelium composed of chitin, cellulose, 15 glucan, chitosan, mannan, and other complex polysaccharides. Vegetative growth is by hyphal elongation and carbon catabolism is obligately aerobic. In contrast, vegetative growth by yeasts such as Saccharomyces cerevisiae is by budding of a unicellular thallus and carbon catabolism may be fermentative. In a more 20 preferred embodiment, the filamentous fungal host cell is a cell of a species of, but not limited to, Acremonium, Aspergillus, Fusarium, Humicola, Mucor, Myceliophthora, Neurospora, Penicillium, Thielavia, Tolypocladium, and Trichoderma or a teleomorph or synonym thereof. In an even more preferred embodiment, the 25 filamentous fungal host cell is an Aspergillus cell. In another even more preferred embodiment, the filamentous fungal host cell is an Acremonium cell. In another even more preferred embodiment, the filamentous fungal host cell is a Fusarium cell. In another even more preferred embodiment, the filamentous fungal 30 host cell is a Humicola cell. In another even more preferred embodiment, the filamentous fungal host cell is a Mucor cell. In another even more preferred embodiment, the filamentous fungal host cell is a Myceliophthora cell. In another even more preferred embodiment, the filamentous fungal host cell is a Neu-

rospora cell. In another even more preferred embodiment, the filamentous fungal host cell is a *Penicillium* cell. In another even more preferred embodiment, the filamentous fungal host cell is a *Thielavia* cell. In another even more preferred embodiment, the filamentous fungal host cell is a *Tolypocladium* cell. In another even more preferred embodiment, the filamentous fungal host cell is a *Trichoderma* cell. In a most preferred embodiment, the filamentous fungal host cell is an *Aspergillus* *awamori*, *Aspergillus foetidus*, *Aspergillus japonicus*, *Aspergillus niger*, *Aspergillus nidulans* or *Aspergillus oryzae* cell. In another most preferred embodiment, the filamentous fungal host cell is a *Fusarium* cell of the section *Discolor* (also known as the section *Fusarium*). For example, the filamentous fungal parent cell may be a *Fusarium bactridioides*, *Fusarium cerealis*, *Fusarium crookwellense*, *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium graminum*, *Fusarium heterosporum*, *Fusarium negundi*, *Fusarium reticulatum*, *Fusarium roseum*, *Fusarium sambucinum*, *Fusarium sarcochroum*, *Fusarium sulphureum*, or *Fusarium trichothecioides* cell. In another preferred embodiment, the filamentous fungal parent cell is a *Fusarium* strain of the section *Elegans*, e.g., *Fusarium oxysporum*. In another most preferred embodiment, the filamentous fungal host cell is a *Humicola insolens* or *Humicola lanuginosa* cell. In another most preferred embodiment, the filamentous fungal host cell is a *Mucor miehei* cell. In another most preferred embodiment, the filamentous fungal host cell is a *Myceliophthora thermophilum* cell. In another most preferred embodiment, the filamentous fungal host cell is a *Neurospora crassa* cell. In another most preferred embodiment, the filamentous fungal host cell is a *Penicillium purpurogenum* cell. In another most preferred embodiment, the filamentous fungal host cell is a *Thielavia terrestris* cell or a *Acremonium chrysogenum* cell. In another most preferred embodiment, the *Trichoderma* cell is a *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma longibrachiatum*, *Trichoderma reesei*

or *Trichoderma viride* cell. The use of *Aspergillus* spp. for the expression of proteins is described in, e.g., EP 272 277, EP 230 023.

5 Transformation

Fungal cells may be transformed by a process involving protoplast formation, transformation of the protoplasts, and regeneration of the cell wall in a manner known per se. Suitable procedures for transformation of *Aspergillus* host cells are described in EP 238 023 and Yelton et al., 1984, Proceedings of the National Academy of Sciences USA 81:1470-1474. A suitable method of transforming *Fusarium* species is described by Malardier et al., 1989, Gene 78:147-156 or in copending US Serial No. 08/269,449. Examples of other fungal cells are cells of filamentous fungi, e.g. *Aspergillus* spp., *Neurospora* spp., *Fusarium* spp. or *Trichoderma* spp., in particular strains of *A. oryzae*, *A. nidulans* or *A. niger*. The use of *Aspergillus* spp. for the expression of proteins is described in, e.g., EP 272 277, EP 230 023, EP 184 ... The transformation of *F. oxysporum* may, for instance, be carried out as described by Malardier et al., 1989, Gene 78: 147-156.

Yeast may be transformed using the procedures described by Becker and Guarente, In Abelson, J.N. and Simon, M.I., editors, Guide to Yeast Genetics and Molecular Biology, Methods in Enzymology, Volume 194, pp 182-187, Academic Press, Inc., New York; Ito et al., 1983, Journal of Bacteriology 153:163; and Hinnen et al., 1978, Proceedings of the National Academy of Sciences USA 75:1920. Mammalian cells may be transformed by direct uptake using the calcium phosphate precipitation method of Graham and Van der Eb (1978, Virology 52:546).

Transformation of insect cells and production of heterologous polypeptides therein may be performed as described in US 4,745,051; US 4, 775, 624; US 4,879,236; US 5,155,037; US

5,162,222; EP 397,485) all of which are incorporated herein by reference. The insect cell line used as the host may suitably be a Lepidoptera cell line, such as *Spodoptera frugiperda* cells or *Trichoplusia ni* cells (cf. US 5,077,214). Culture conditions may
5 suitably be as described in, for instance, WO 89/01029 or WO 89/01028, or any of the aforementioned references.

Methods of Production

- 10 The transformed or transfected host cells described above are cultured in a suitable nutrient medium under conditions permitting the production of the desired molecules, after which these are recovered from the cells, or the culture broth.
- 15 The medium used to culture the cells may be any conventional medium suitable for growing the host cells, such as minimal or complex media containing appropriate supplements. Suitable media are available from commercial suppliers or may be prepared according to published recipes (e.g. in catalogues of the American
20 Type Culture Collection). The media are prepared using procedures known in the art (see, e.g., references for bacteria and yeast; Bennett, J.W. and LaSure, L., editors, *More Gene Manipulations in Fungi*, Academic Press, CA, 1991).
- 25 If the molecules are secreted into the nutrient medium, they can be recovered directly from the medium. If they are not secreted, they can be recovered from cell lysates. The molecules are recovered from the culture medium by conventional procedures including separating the host cells from the medium by centrifuga-
30 tion or filtration, precipitating the proteinaceous components of the supernatant or filtrate by means of a salt, e.g. ammonium sulphate, purification by a variety of chromatographic procedures, e.g. ion exchange chromatography, gel filtration chroma-

tography, affinity chromatography, or the like, dependent on the type of molecule in question.

The molecules of interest may be detected using methods known in the art that are specific for the molecules. These detection methods may include use of specific antibodies, formation of a product, or disappearance of a substrate. For example, an enzyme assay may be used to determine the activity of the molecule. Procedures for determining various kinds of activity are known in the art.

The molecules of the present invention may be purified by a variety of procedures known in the art including, but not limited to, chromatography (e.g., ion exchange, affinity, hydrophobic, chromatofocusing, and size exclusion), electrophoretic procedures (e.g., preparative isoelectric focusing (IEF), differential solubility (e.g., ammonium sulfate precipitation), or extraction (see, e.g., Protein Purification, J-C Janson and Lars Ryden, editors, VCH Publishers, New York, 1989).

20

The term "immunological response", used in connection with the present invention, is the response of an organism to a compound, which involves the immune system according to any of the four standard reactions (Type I, II, III and IV according to Coombs & Gell).

25

Correspondingly, the "immunogenicity" of a compound used in connection with the present invention refers to the ability of this compound to induce an 'immunological response' in animals including man.

30

The term "allergic response", used in connection with the present invention, is the response of an organism to a compound, which involves IgE mediated responses (Type I reaction according

to Coombs & Gell). It is to be understood that sensitization (i.e. development of compound-specific IgE antibodies) upon exposure to the compound is included in the definition of "allergic response".

5

Correspondingly, the "allergenicity" of a compound used in connection with the present invention refers to the ability of this compound to induce an 'allergic response' in animals including man.

10

The term "parent protein" refer to the polypeptide to be modified by creating a library of diversified mutants. The "parent protein" may be a naturally occurring (or wild-type) polypeptide or it may be a variant thereof prepared by any suitable means.

15 For instance, the "parent protein" may be a variant of a naturally occurring polypeptide which has been modified by substitution, deletion or truncation of one or more amino acid residues or by addition or insertion of one or more amino acid residues to the amino acid sequence of a naturally-occurring polypeptide.

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The term "enzyme variants" or "protein variants" refer to a polypeptide of the invention comprising one or more substitutions of the specified amino acid residues. The total number of such substitutions is typically not more than 10, e.g. one, two, 25 three, four, five or six of said substitutions. In addition, the enzyme variant or protein variant of the invention may optionally include other modifications of the parent enzyme, typically not more than 10, e.g. not more than 5 such modifications. The variant generally has a homology with the parent enzyme of at 30 least 80 %, e.g. at least 85 %, typically at least 90 % or at least 95 %.

The term " randomized library" of protein variants refers to a library with at least partially randomized composition of the members, e.g. protein variants.

5 An "epitope" is a set of amino acids on a protein that are involved in an immunological response, such as antibody binding or T-cell activation. One particularly useful method of identifying epitopes involved in antibody binding is to screen a library of peptide-phage membrane protein fusions and selecting those that
10 bind to relevant antigen-specific antibodies, sequencing the randomized part of the fusion gene, aligning the sequences involved in binding, defining consensus sequences based on these alignments, and mapping these consensus sequences on the surface or the sequence and/or structure of the antigen, to identify
15 epitopes involved in antibody binding.

By the term "epitope pattern" is meant such a consensus sequence of antibody binding peptides. An example is the epitope pattern A R R < R. The sign "<" in this notation indicates that the
20 aligned antibody binding peptides included a non-consensus amino acid between the second and the third arginine.

An "epitope area" is defined as the amino acids situated close to the epitope sequence amino acids. Preferably, the amino acids
25 of an epitope area are located <5Å from the epitope sequence. Hence, an epitope area also includes the corresponding epitope sequence itself. Modifications of amino acids of the 'epitope area' can possibly affect the immunogenic function of the corresponding epitope.

30

By the term "epitope sequence" is meant the amino acid residues of a parent protein, which have been identified to belong to an epitope by the methods of the present invention (an example of an epitope sequence is E271 Q12 I8 in Savinase).

The term 'antibody binding peptide' denotes a peptide that bind with sufficiently high affinity to antibodies. Identification of 'antibody binding peptides' and their sequences constitute the first step of the method of this invention.

"Anchor amino acids" are the individual amino acids of an epitope pattern.

10 "Hot spot amino acids" are amino acids of parent protein, which are particularly likely to result in modified immunogenecity if they are mutated. Amino acids, which appear in three or more epitope sequences or which correspond to anchor amino acids are hot spot amino acids.

15

"Environmental allergens" are protein allergens that are present naturally. They include pollen, dust mite allergens, pet allergens, food allergens, venoms, etc.

20 "Commercial allergens" are protein allergens that are being brought to the market commercially. They include enzymes, pharmaceutical proteins, antimicrobial peptides, as well as allergens of transgenic plants.

25 The "donor protein" is the protein that was used to raise antibodies used to identify antibody binding sequences, hence the donor protein provides the information that leads to the epitope patterns.

30 The "acceptor protein" is the protein, whose structure is used to fit the identified epitope patterns and/or to fit the antibody binding sequences. Hence the acceptor protein is also the parent protein.

An "autoepitope" is one that has been identified using antibodies raised against the parent protein, i.e. the acceptor and the donor proteins are identical.

- 5 A "heteroepitope" is one that has been identified with distinct donor and acceptor proteins.

The term "functionality" of protein variants refers to e.g. enzymatic activity; binding to a ligand or receptor; stimulation
10 of a cellular response (e.g. ³H-thymidine incorporation as response to a mitogenic factor); or anti-microbial activity.

By the term "specific polyclonal antibodies" is meant polyclonal antibodies isolated according to their specificity for a certain
15 antigen, e.g. the protein backbone.

By the term "monospecific antibodies" is meant polyclonal antibodies isolated according to their specificity for a certain epitope. Such monospecific antibodies will bind to the same epitope,
20 tope, but with different affinity, as they are produced by a number of antibody producing cells recognizing overlapping but not necessarily identical epitopes.

The term "randomized library" of protein variants refers to a
25 library with at least partially randomized composition of the members, e.g. protein variants.

'Spiked mutagenesis' is a form of site-directed mutagenesis, in which the primers used have been synthesized using mixtures of
30 oligonucleotides at one or more positions.

By the term "a protein variant having modified immunogenicity as compared to the parent protein" is meant a protein variant which differs from the parent protein in one or more amino acids

whereby the immunogenicity of the variant is modified. The modification of immunogenicity may be confirmed by testing the ability of the protein variant to elicit an IgE/IgG response.

5 In the present context the term "protein" is intended to cover oligopeptides, polypeptides as well as proteins as such.

10 Detailed description of the invention

The present invention relates to a method of selecting a protein variant having modified immunogenicity as compared to a parent protein,

15

comprising the steps of:

- a) obtaining antibody binding peptide sequences,
- 20 b) using the sequences to localise epitope sequences on the 3-dimensional structure of parent protein,
- c) defining an epitope area including amino acids situated within 5 Å from the epitope amino acids constituting the epitope
25 sequence,
- d) changing one or more of the amino acids defining the epitope area of the parent protein by genetic engineering mutations of a DNA sequence encoding the parent protein,
- 30 e) introducing the mutated DNA sequence into a suitable host, culturing said host and expressing the protein variant, and

f) evaluating the immunogenicity of the protein variant using the parent protein as reference.

5 A) How to find antibody binding peptide sequences and epitope patterns

A first step of the method is to identify peptide sequences, which bind specifically to antibodies.

10

Antibody binding peptide sequences can be found by testing a set of known peptide sequences for binding to antibodies raised against the donor protein. These sequences are typically selected, such that each represents a segment of the donor protein
15 sequence (Mol. Immunol., 1992, vol. 29, pp.1383-1389; Am. J. Resp. Cell, Mol. Biol. 2000, vol. 22, pp. 344-351). Also, randomized synthetic peptide libraries can be used to find antibody binding sequences (Slootstra et al; Molecular Diversity, 1996, vol. 2, pp. 156-164).

20

In a preferred method, the identification of antibody binding sequences may be achieved by screening of a display package library, preferably a phage display library. The principle behind
25 phage display is that a heterologous DNA sequence can be inserted in the gene coding for a coat protein of the phage (WO 92/15679). The phage will make and display the hybrid protein on its surface where it can interact with specific target agents. Such target agent may be antigen-specific antibodies. It is
30 therefore possible to select specific phages that display antibody-binding peptide sequences. The displayed peptides can be of predetermined lengths, for example 9 amino acids long, with randomized sequences, resulting in a random peptide display package library. Thus, by screening for antibody binding, one can iso-

late the peptide sequences that have sufficiently high affinity for the particular antibody used. The peptides of the hybrid proteins of the specific phages which bind protein-specific antibodies characterize epitopes that are recognized by the immune
5 system.

The antibodies used for reacting with the display package are preferably IgE antibodies to ensure that the epitopes identified are IgE epitopes, i.e. epitopes inducing and binding IgE. In a
10 preferred embodiment the antibodies are polyclonal antibodies, optionally monospecific antibodies.

For the purpose of the present invention polyclonal antibodies are preferred in order to obtain a broader knowledge about the
15 epitopes of a protein.

It is of great importance that the amino acid sequence of the peptides presented by the display packages is long enough to represent a significant part of the epitope to be identified. In
20 a preferred embodiment of the invention the peptides of the peptide display package library are oligopeptides having from 5 to 25 amino acids, preferably at least 8 amino acids, such as 9 amino acids. For a given length of peptide sequences (n), the theoretical number of different possible sequences can be calcu-
25 lated as 20^n . The diversity of the package library used must be large enough to provide a suitable representation of the theoretical number of different sequences. In a phage-display library, each phage has one specific sequence of a determined length. Hence an average phage display library can express 10^8 -
30 10^{12} different random sequences, and is therefore well-suited to represent the theoretical number of different sequences.

The antibody binding peptide sequences can be further analysed by consensus alignment e.g. by the methods described by Feng and

Doolittle, Meth. Enzymol., 1996, vol. 266, pp. 368-382; Feng and Doolittle, J. Mol. Evol., 1987, vol. 25, pp. 351-360; and Taylor, Meth. Enzymol., 1996, vol. 266, pp. 343-367.

5

This leads to identification of epitope patterns, which can assist the comparison of the linear information obtained from the antibody binding peptide sequences to the 3-dimensional structure of the acceptor protein in order to identify epitope sequences at the surface of the acceptor protein.

B) How to identify epitope sequences and epitope areas.

15 Given a number of antibody binding peptide sequences and possibly the corresponding epitope patterns, one need the 3-dimensional structure coordinates of an acceptor protein to find the epitope sequences on its surface.

20 These coordinates can be found in databases (NCBI: <http://www.ncbi.nlm.nih.gov/>), determined experimentally using conventional methods (Ducruix and Giegé: Crystallization of Nucleic Acids and Proteins, IRL Press, Oxford, 1992, ISBN 0-19-963245-6), or they can be deduced from the coordinates of a homologous protein. Typical actions required for the construction of a model structure are: alignment of homologous sequences for which 3-dimensional structures exist, definition of Structurally Conserved Regions (SCRs), assignment of coordinates to SCRs, search for structural fragments/loops in structure databases to replace Variable Regions, assignment of coordinates to these regions, and structural refinement by energy minimization. Regions containing large inserts (>3 residues) relative to the known 3-dimensional structures are known to be quite difficult

to model, and structural predictions must be considered with care.

Using the coordinates and the several methods of mapping the linear information on the 3-dimensional surface are possible, as described in the examples below.

One can match each amino acid residue of the antibody binding peptide to an identical or homologous amino acid on the 3-D surface of the acceptor protein, such that amino acids that are adjacent in the primary sequence are close on the surface of the acceptor protein, with close being $<5\text{\AA}$, preferably $<3\text{\AA}$ between any two atoms of the two amino acids.

Alternatively, one can define a geometric body (e.g. an ellipsoid, a sphere, or a box) of a size that matches a possible binding interface between antibody and antigen and look for a positioning of this body where it will contain most of or all the anchor amino acids.

Also, one can use the epitope patterns to facilitate identification of epitope sequences. This can be done, by first matching the anchor amino acids on the 3-D structure and subsequently looking for other elements of the antibody binding peptide sequences, which provide additional matches. If there are many residues to be matched, it is only necessary that a suitable number can be found on the 3-D structure. For example if an epitope pattern comprises 4, 5, 6, or 7 amino acids, it is only necessary that 3 matches surface elements of the acceptor protein.

In all cases, it is desirable that amino acids of the epitope sequence are surface exposed (as described below in Examples).

It is known, that amino acids that surround binding sequences can affect binding of a ligand without participating actively in the binding process. Based on this knowledge, areas covered by amino acids with potential steric effects on the epitope-
5 antibody interaction, were defined around the identified epitope sequences. These areas are called 'epitope areas'. Practically, all amino acids situated within 5Å from the amino acids defining the epitope sequence were included. Preferably, the epitope area equals the epitope sequence. The accessibility criterium was not
10 used as hidden amino acids of an epitope area also can have an effect on the adjacent amino acids of the epitope sequence.

C) How to use the epitope information.

15

There are at least four ways to utilize the information about epitope sequences, which has been derived by the methods of this invention:

20 1) reduce the allergenicity of a commercial protein using protein engineering.

2) reduce the potential of commercial proteins to cross-react with environmental allergens and hence cause allergic reactions in people sensitized to the environmental allergens (or vice
25 versa).

3) improve the immunotherapeutic effect of allergen vaccines.

4) assist characterization of clinical allergies in order to select the appropriate allergen vaccine.

30

Protein engineering to reduce the allergenicity, cross-reactivity and/or immunotherapeutic effect of proteins.

The methods described thus far have led to identification of epitope areas on an acceptor protein, each containing epitope sequences. These subsets of amino acids, are preferred for introducing mutations that are meant to modify the immunogenecity of the acceptor protein. An even more preferred subset of amino acids to target by mutagenesis are 'hot spot amino acids', which appear in several different epitope sequences, or which corresponds to anchor amino acids of the epitope patterns. Thus, genetic engineering mutations should be designed in the epitope areas, preferably in epitope sequences, and more preferably in the 'hot spot amino acids'.

Substitution, deletion, insertion

15

When the epitope area(s) have been identified, a protein variant exhibiting a modified immunogenicity may be produced by changing the identified epitope area of the parent protein by genetic engineering mutation of a DNA sequence encoding the parent protein.

20

The epitope identified may be changed by substituting at least one amino acid of the epitope area. In a preferred embodiment at least one anchor amino acid or hot spot amino acid is changed. The change will often be substituting to an amino acid of different size, hydrophilicity, and/or polarity, such as a small amino acid versus a large amino acid, a hydrophilic amino acid versus a hydrophobic amino acid, a polar amino acid versus a non-polar amino acid and a basic versus an acidic amino acid.

30

Other changes may be the addition/insertion or deletion of at least one amino acid of the epitope sequence, preferably deleting an anchor amino acid or a hot spot amino acid. Furthermore,

an epitope pattern may be changed by substituting some amino acids, and deleting/adding other.

In the claims a position to be changed by substitution, insertion, deletion will be indicated by: "Position xx to aaa, bbb, ccc, insertion, deletion", meaning that position xx can be substituted by the amino acid aaa, bbb, ccc or that any amino acid can be inserted after position xx or that position xx can be deleted, e.g. "Position 27 to A, D, E, insertion, deletion" means
10 that in position 27 the amino acid can be substituted by A, D or E, or that any amino acid can be inserted after position 27, or that the amino acid in position 27 can be deleted.

When one uses protein engineering to eliminate epitopes, it is
15 indeed possible that new epitopes are created, or existing epitopes are duplicated. To reduce this risk, one can map the planned mutations at a given position on the 3-dimensional structure of the protein of interest, and control the emerging amino acid constellation against a database of known epitope
20 patterns, to rule out those possible replacement amino acids, which are predicted to result in creation or duplication of epitopes. Thus, risk mutations can be identified and eliminated by this procedure, thereby reducing the risk of making mutations that lead to increased rather than decreased allergenicity.

25

Introduction of residues for chemical derivatization in epitope areas

30 In yet another embodiment, one can design the mutation, such that amino acids suitable for chemical modification are substituted for existing ones in the epitope areas. The protein variant can then be conjugated to activated polymers. Which amino acids to substitute and/or insert, depends in principle on the

coupling chemistry to be applied. The chemistry for preparation of covalent bioconjugates can be found in "Bioconjugate Techniques", Hermanson, G.T. (1996), Academic Press Inc., which is hereby incorporated as reference (see below). It is preferred to make conservative substitutions in the polypeptide when the polypeptide has to be conjugated, as conservative substitutions secure that the impact of the substitution on the polypeptide structure is limited. In the case of providing additional amino groups this may be done by substitution of arginine to lysine, both residues being positively charged, but only the lysine having a free amino group suitable as an attachment groups. In the case of providing additional carboxylic acid groups the conservative substitution may for instance be an asparagine to aspartic acid or glutamine to glutamic acid substitution. These residues resemble each other in size and shape, except from the carboxylic groups being present on the acidic residues. In the case of providing SH-groups the conservative substitution may be done by changing threonine or serine to cysteine.

20

Chemical conjugation

For chemical conjugation, the protein variant needs to be incubate with an active or activated polymer and subsequently separated from the unreacted polymer. This can be done in solution followed by purification or it can conveniently be done using the immobilized protein variants, which can easily be exposed to different reaction environments and washes.

In the case where polymeric molecules are to be conjugated with the polypeptide in question and the polymeric molecules are not active they must be activated by the use of a suitable technique. It is also contemplated according to the invention to couple the polymeric molecules to the polypeptide through a

linker. Suitable linkers are well-known to the skilled person. Methods and chemistry for activation of polymeric molecules as well as for conjugation of polypeptides are intensively described in the literature. Commonly used methods for activation of insoluble polymers include activation of functional groups with cyanogen bromide, periodate, glutaraldehyde, biepoxydes, epichlorohydrin, divinylsulfone, carbodiimide, sulfonyl halides, trichlorotriazine etc. (see R.F. Taylor, (1991), "Protein immobilisation. Fundamental and applications", Marcel Dekker, N.Y.; S.S. Wong, (1992), "Chemistry of Protein Conjugation and Crosslinking", CRC Press, Boca Raton; G.T. Hermanson et al., (1993), "Immobilized Affinity Ligand Techniques", Academic Press, N.Y.). Some of the methods concern activation of insoluble polymers but are also applicable to activation of soluble polymers e.g. periodate, trichlorotriazine, sulfonylhalides, divinylsulfone, carbodiimide etc. The functional groups being amino, hydroxyl, thiol, carboxyl, aldehyde or sulfhydryl on the polymer and the chosen attachment group on the protein must be considered in choosing the activation and conjugation chemistry which normally consist of i) activation of polymer, ii) conjugation, and iii) blocking of residual active groups.

In the following a number of suitable polymer activation methods will be described shortly. However, it is to be understood that also other methods may be used.

Coupling polymeric molecules to the free acid groups of polypeptides may be performed with the aid of diimide and for example amino-PEG or hydrazino-PEG (Pollak et al., (1976), J. Am. Chem. Soc., 98, 289-291) or diazoacetate/amide (Wong et al., (1992), "Chemistry of Protein Conjugation and Crosslinking", CRC Press).

Coupling polymeric molecules to hydroxy groups is generally very difficult as it must be performed in water. Usually hydrolysis predominates over reaction with hydroxyl groups.

5 Coupling polymeric molecules to free sulfhydryl groups can be achieved with special groups like maleimido or the ortho-pyridyl disulfide. Also vinylsulfone (US patent no. 5,414,135, (1995), Snow et al.) has a preference for sulfhydryl groups but is not as selective as the other mentioned.

10

Accessible arginine residues in the polypeptide chain may be targeted by groups comprising two vicinal carbonyl groups.

Techniques involving coupling of electrophilically activated
15 PEGs to the amino groups of Lysines may also be useful. Many of the usual leaving groups for alcohols give rise to an amine linkage. For instance, alkyl sulfonates, such as tresylates (Nilsson et al., (1984), Methods in Enzymology vol. 104, Jacoby, W. B., Ed., Academic Press: Orlando, p. 56-66; Nilsson et al.,
20 (1987), Methods in Enzymology vol. 135; Mosbach, K., Ed.; Academic Press: Orlando, pp. 65-79; Scouten et al., (1987), Methods in Enzymology vol. 135, Mosbach, K., Ed., Academic Press: Orlando, 1987; pp 79-84; Crossland et al., (1971), J. Amr. Chem. Soc. 1971, 93, pp. 4217-4219), mesylates (Harris, (1985), supra;
25 Harris et al., (1984), J. Polym. Sci. Polym. Chem. Ed. 22, pp 341-352), aryl sulfonates like tosylates, and para-nitrobenzene sulfonates can be used.

Organic sulfonyl chlorides, e.g. Tresyl chloride, effectively
30 converts hydroxy groups in a number of polymers, e.g. PEG, into good leaving groups (sulfonates) that, when reacted with nucleophiles like amino groups in polypeptides allow stable linkages to be formed between polymer and polypeptide. In addition to high conjugation yields, the reaction conditions are in general

mild (neutral or slightly alkaline pH, to avoid denaturation and little or no disruption of activity), and satisfy the non-destructive requirements to the polypeptide.

5 Tosylate is more reactive than the mesylate but also less stable decomposing into PEG, dioxane, and sulfonic acid (Zalipsky, (1995), Bioconjugate Chem., 6, 150-165). Epoxides may also been used for creating amine bonds but are much less reactive than the abovementioned groups.

10

Converting PEG into a chloroformate with phosgene gives rise to carbamate linkages to Lysines. Essentially the same reaction can be carried out in many variants substituting the chlorine with N-hydroxy succinimide (US patent no. 5,122,614, (1992); Zalipsky
15 et al., (1992), Biotechnol. Appl. Biochem., 15, p. 100-114; Monfardini et al., (1995), Bioconjugate Chem., 6, 62-69, with imidazole (Allen et al., (1991), Carbohydr. Res., 213, pp 309-319), with para-nitrophenol, DMAP (EP 632 082 A1, (1993), Looze, Y.) etc. The derivatives are usually made by reacting the chloroformate
20 with the desired leaving group. All these groups give rise to carbamate linkages to the peptide.

Furthermore, isocyanates and isothiocyanates may be employed, yielding ureas and thioureas, respectively.

25

Amides may be obtained from PEG acids using the same leaving groups as mentioned above and cyclic imid thrones (US patent no. 5,349,001, (1994), Greenwald et al.). The reactivity of these compounds are very high but may make the hydrolysis to fast.

30

PEG succinate made from reaction with succinic anhydride can also be used. The hereby comprised ester group make the conjugate much more susceptible to hydrolysis (US patent no.

5,122,614, (1992), Zalipsky). This group may be activated with N-hydroxy succinimide.

Furthermore, a special linker can be introduced. The most well studied being cyanuric chloride (Abuchowski et al., (1977), J. Biol. Chem., 252, 3578-3581; US patent no. 4,179,337, (1979), Davis et al.; Shafer et al., (1986), J. Polym. Sci. Polym. Chem. Ed., 24, 375-378.

10 Coupling of PEG to an aromatic amine followed by diazotation yields a very reactive diazonium salt, which can be reacted with a peptide in situ. An amide linkage may also be obtained by reacting an azlactone derivative of PEG (US patent no. 5,321,095, (1994), Greenwald, R. B.) thus introducing an additional amide
15 linkage.

As some peptides do not comprise many Lysines it may be advantageous to attach more than one PEG to the same Lysine. This can be done e.g. by the use of 1,3-diamino-2-propanol.

20 PEGs may also be attached to the amino-groups of the enzyme with carbamate linkages (WO 95/11924, Greenwald et al.). Lysine residues may also be used as the backbone.

The coupling technique used in the examples is the N-succinimidyl carbonate conjugation technique described in WO
25 90/13590 (Enzon).

In a preferred embodiment, the activated polymer is methyl-PEG which has been activated by N-succinimidyl carbonate as described WO 90/13590. The coupling can be carried out at alkaline
30 conditions in high yields.

For coupling of polymers to the protein variants, it is preferred to use conditions similar to those described in WO96/17929 and WO99/00489 (Novo Nordisk A/S) e.g. mono or bis

activated PEG's of molecular weight ranging from 100 to 5000 Da. For instance, a methyl-PEG 350 could be activated with N-succinimidyl carbonate and incubated with protein variant at a molar ratio of more than 5 calculated as equivalents of activated PEG divided by moles of lysines in the protein of interest. For coupling to immobilized protein variant, the PEG:protein ratio should be optimized such that the PEG concentration is low enough for the buffer capacity to maintain alkaline pH throughout the reaction; while the PEG concentration is still high enough to ensure sufficient degree of modification of the protein. Further, it is important that the activated PEG is kept at conditions that prevent hydrolysis (i.e. dissolved in acid or solvents) and diluted directly into the alkaline reaction buffer. It is essential that primary amines are not present other than those occurring in the lysine residues of the protein. This can be secured by washing thoroughly in borate buffer. The reaction is stopped by separating the fluid phase containing unreacted PEG from the solid phase containing protein and derivatized protein. Optionally, the solid phase can then be washed with tris buffer, to block any unreacted sites on PEG chains that might still be present.

Introduction of consensus sequences for post-translational modifications in the epitope areas

In another embodiment, the mutations are designed, such that recognition sites for post-translational modifications are introduced in the epitope areas, and the protein variant is expressed in a suitable host organism capable of the corresponding post-translational modification. These post-translational modifications may serve to shield the epitope and hence lower the immunogenicity of the protein variant relative to the protein backbone. Post-translational modifications include glycosyla-

tion, phosphorylation, N-terminal processing, acylation, ribosylation and sulfatation. A good example is N-glycosylation. N-glycosylation is found at sites of the sequence Asn-Xaa-Ser, Asn-Xaa-Thr, or Asn-Xaa-Cys, in which neither the Xaa residue nor the amino acid following the tri-peptide consensus sequence is a proline (T. E. Creighton, 'Proteins - Structures and Molecular Properties, 2nd edition, W.H. Freeman and Co., New York, 1993, pp. 91-93). It is thus desirable to introduce such recognition sites in the sequence of the backbone protein. The specific nature of the glycosyl chain of the glycosylated protein variant may be linear or branched depending on the protein and the host cells. Another example is phosphorylation: The protein sequence can be modified so as to introduce serine phosphorylation sites with the recognition sequence arg-arg-(xaa)_n-ser (where n = 0, 1, or 2), which can be phosphorylated by the cAMP-dependent kinase or tyrosine phosphorylation sites with the recognition sequence -lys/arg - (xaa)₃ - asp/glu- (xaa)₃ - tyr, which can usually be phosphorylated by tyrosine-specific kinases (T.E. Creighton, "Proteins- Structures and molecular properties", 2nd ed., Freeman, NY, 1993).

Randomized approaches to introduce modifications in epitope areas.

25

In order to generate protein variants, more than one amino acid residue may be substituted, added or deleted, these amino acids preferably being located in different epitope areas. In that case, it may be difficult to assess a priori how well the functionality of the protein is maintained while antigenicity is reduced, especially since the possible number of mutation-combinations becomes very large, even for a small number of mutations. In that case, it will be an advantage, to establish a library of diversified mutants each having one or more changed

amino acids introduced and selecting those variants, which show good retention of function and at the same time a significant reduction in antigenicity.

5 A diversified library can be established by a range of techniques known to the person skilled in the art (Reetz MT; Jaeger KE, in 'Biocatalysis - from Discovery to Application' edited by Fessner WD, Vol. 200, pp. 31-57 (1999); Stemmer, Nature, vol. 370, p.389-391, 1994; Zhao and Arnold, Proc. Natl. Acad. Sci.,
10 USA, vol. 94, pp. 7997-8000, 1997; or Yano et al., Proc. Natl. Acad. Sci., USA, vol. 95, pp 5511-5515, 1998). These include, but are not limited to, 'spiked mutagenesis', in which certain positions of the protein sequence are randomized by carrying out PCR mutagenesis using one or more oligonucleotide primers which
15 are synthesized using a mixture of nucleotides for certain positions (Lanio T, Jeltsch A, Biotechniques, Vol. 25(6), 958,962,964-965 (1998)). The mixtures of oligonucleotides used within each triplet can be designed such that the corresponding amino acid of the mutated gene product is randomized within some
20 predetermined distribution function. Algorithms have been disclosed, which facilitate this design (Jensen LJ et al., Nucleic Acids Research, Vol. 26(3), 697-702 (1998)).

In an embodiment substitutions are found by a method comprising
25 the following steps: 1) a range of substitutions, additions, and/or deletions are listed encompassing several epitope areas (preferably in the corresponding epitope sequences, anchor amino acids, and/or hot spots), 2) a library is designed which introduces a randomized subset of these changes in the amino acid se-
30 quence into the target gene, e.g. by spiked mutagenesis, 3) the library is expressed, and preferred variants are selected. In another embodiment, this method is supplemented with additional rounds of screening and/or family shuffling of hits from the first round of screening (J.E. Ness, et al, Nature Biotechnol-

ogy, vol. 17, pp. 893-896, 1999) and/or combination with other methods of reducing immunogenicity by genetic means (such as that disclosed in WO92/10755).

5 The library may be designed, such that at least one amino acid of the epitope area is substituted. In a preferred embodiment at least one amino acid of the epitope sequence itself is changed, and in an even more preferred embodiment, one or more hot spot amino acids are changed. The library may be biased such that to-
10 wards introducing an amino acid of different size, hydrophilicity, and/or polarity relative to the original one of the 'protein backbone'. For example changing a small amino acid to a large amino acid, a hydrophilic amino acid to a hydrophobic amino acid, a polar amino acid to a non-polar amino acid or a
15 basic to an acidic amino acid. Other changes may be the addition or deletion of at least one amino acid of the epitope area, preferably deleting an anchor amino acid. Furthermore, substituting some amino acids and deleting or adding others may change an epitope.

20

Diversity in the protein variant library can be generated at the DNA triplet level, such that individual codons are variegated e.g. by using primers of partially randomized sequence for a PCR reaction. Further, several techniques have been described, by
25 which one can create a library with such diversity at several locations in the gene, which are too far apart to be covered by a single (spiked) oligonucleotide primer. These techniques include the use of in vivo recombination of the individually diversified gene segments as described in WO 97/07205 on page 3,
30 line 8 to 29 or by using DNA shuffling techniques to create a library of full length genes that combine several gene segments each of which are diversified e.g. by spiked mutagenesis (Stemmer, Nature 370, pp. 389-391, 1994 and US 5,605,793 and 5,830,721). In the latter case, one can use the gene encoding

the "protein backbone" as a template double-stranded polynucleotide and combining this with one or more single or double-stranded oligonucleotides as described in claim 1 of US 5,830,721. The single-stranded oligonucleotides could be partially randomized during synthesis. The double-stranded oligonucleotides could be PCR products incorporating diversity in a specific region. In both cases, one can dilute the diversity with corresponding segments containing the sequence of the backbone protein in order to limit the number of changes that are on average introduced. As mentioned above, methods have been established for designing the ratios of nucleotides (A; C; T; G) used at a particular codon during primer synthesis, so as to approximate a desired frequency distribution among a set of desired amino acids at that particular codon. This allows one to bias the partially randomized mutagenesis towards e.g. introduction of post-translational modification sites, chemical modification sites, or simply amino acids that are different from those that define the epitope or the epitope area. One could also approximate a sequence in a given location or epitope area to the corresponding location on a homologous, human protein.

Occasionally, one would be interested in testing a library that combines a number of known mutations in different locations in the primary sequence of the 'protein backbone'. These could be introduced post-translational or chemical modification sites, or they could be mutations, which by themselves had proven beneficial for one reason or another (e.g. decreasing antigenicity, or improving specific activity, performance, stability, or other characteristics). In such cases, it may be desirable to create a library of diverse combinations of known sequences. For example if 12 individual mutations are known, one could combine (at least) 12 segments of the 'protein backbone' gene in which each segment is present in two forms: one with and one without the desired mutation. By varying the relative amounts of those seg-

ments, one could design a library (of size 2^{12}) for which the average number of mutations per gene can be predicted. This can be a useful way of combining elements that by themselves give some, but not sufficient effect, without resorting to very large libraries, as is often the case when using 'spiked mutagenesis'. Another way to combine these 'known mutations' could be by using family shuffling of oligomeric DNA encoding the known changes with fragments of the full length wild type sequence.

10

Assays for reduced allergenicity

When protein variants have been constructed based on the methods described in this invention, it is desirable to confirm their antibody binding capacity, functionality, immunogenicity and/or allergenicity using a purified preparation. For that use, the protein variant of interest can be expressed in larger scale, purified by conventional techniques, and the antibody binding and functionality should be examined in detail using dose-response curves and e.g. direct or competitive ELISA (C-ELISA).

The potentially reduced allergenicity (which is likely, but not necessarily true for a variant w. low antibody binding) should be tested in in vivo or in vitro model systems: e.g. an in vitro assays for immunogenicity such as assays based on cytokine expression profiles or other proliferation or differentiation responses of epithelial and other cells incl. B-cells and T-cells. Further, animal models for testing allergenicity should be set up to test a limited number of protein variants that show desired characteristics in vitro. Useful animal models include the guinea pig intratracheal model (GPIT) (Ritz, et al. Fund. Appl. Toxicol., 21, pp. 31-37, 1993), mouse subcutaneous (mouse-SC) (WO 98/30682, Novo Nordisk), the rat intratracheal (rat-IT) (WO 96/17929, Novo Nordisk), and the mouse intranasal (MINT)

(Robinson et al., Fund. Appl. Toxicol. 34, pp. 15-24, 1996) models.

The immunogenicity of the protein variant is measured in animal tests, wherein the animals are immunised with the protein variant and the immune response is measured. Specifically, it is of interest to determine the allergenicity of the protein variants by repeatedly exposing the animals to the protein variant by the intratracheal route and following the specific IgG and IgE titers. Alternatively, the mouse intranasal (MINT) test can be used to assess the allergenicity of protein variants. By the present invention the allergenicity is reduced at least 3 times as compared to the allergenicity of the parent protein, preferably 10 times reduced, more preferably 50 times.

15

However, the present inventors have demonstrated that the performance in ELISA correlates closely to the immunogenic responses measured in animal tests. To obtain a useful reduction of the allergenicity of a protein, the IgE binding capacity of the protein variant must be reduced to at least below 75 %, preferably below 50 %, more preferably below 25 % of the IgE binding capacity of the parent protein as measured by the performance in IgE ELISA, given the value for the IgE binding capacity of the parent protein is set to 100 %.

25

Thus a first assessment of the immunogenicity and/or allergenicity of a protein can be made by measuring the antibody binding capacity or antigenicity of the protein variant using appropriate antibodies. This approach has also been used in the literature (WO 99/47680).

30

Assays for altered immunotherapeutic effect

The immunotherapeutic effect of allergen vaccines can be assessed a number of different ways. One is to measure the specific IgE binding, the reduction of which indicates a better allergen vaccine potential (WO 99/47680, ALK-ABELLÓ). Also, several cellular assays could be employed to show the modified immuneresponse indicative of good allergen vaccine potential as shown in several publications, all of which are hereby incorporated by reference (van Neerven et al, " T lymphocyte responses to allergens: Epitope-specificity and clinical relevance", Immunol Today, 1996, vol. 17, pp. 526-532; Hoffmann et al., Allergy, 1999, vol. 54, pp. 446-454, WO99/07880).

Eventually, clinical trials with allergic patients could be employed using cellular or clinical end-point measurements. (Ebner et al., Clin. Exp. All., 1997, vol. 27, pp. 107-1015; Int. Arch. Allergy Immunol., 1999, vol. 119, pp 1-5).

Determining functionality

20

A wide variety of protein functionality assays are available in the literature. Especially, those suitable for automated analysis are useful for this invention. Several have been published in the literature such as protease assays (WO99/34011, Genencor International; J.E. Ness, et al, Nature Biotechn., 17, pp. 893-896, 1999), oxidoreductase assays (Cherry et al., Nature Biotechn., 17 , pp. 379-384, 1999, and assays for several other enzymes (WO99/45143, Novo Nordisk). Those assays that employ soluble substrates can be employed for direct analysis of functionality of immobilized protein variants.

Cross-reactivity

A related objective is to reduce cross-reactivity between 'commercial allergens' and 'environmental allergens'. Cross-reactivities between food allergens of different origin are well-known (Akkerdaas et al, Allergy 50, pp 215-220, 1995).
5 Similarly, cross-reactivities between other environmental allergens (like pollen, dust mites etc.) and commercial allergens (like enzyme proteins) have been established in the literature (J. All. Clin. Immunol., 1998, vol. 102, pp. 679-686 and by the present inventors. The molecular reason for this cross-
10 reactivity can be explored using epitope mapping. By finding epitope patterns using antibodies raised against environmental allergen (donor protein) and mapping this information on a commercial allergen (the acceptor protein), one may find the epitopes that are common to both proteins, and hence responsible
15 for the cross-reactivity. Obviously, one can also use the commercial allergen as donor and the environmental allergen as acceptor. By modifying the commercial allergen using protein engineering in the epitope areas identified as described above, one can reduce the cross-reactivity of the commercial allergen
20 variant towards the environmental allergens (and vice versa). Hence, the use of the modified commercial allergens would be safer than using the unmodified commercial allergen.

Testing of this approach would be done using an antibody-binding
25 assay with the protein variant (and its parent protein as control) and antibodies raised against the protein that cross-reacts with the parent protein. The method is otherwise identical to those described in the Methods section for characterization of allergenicity and antigenicity.

30

Wash performance etc.

The modifications of the enzymes in the epitope areas as disclosed the present application may cause other effects to the

enzyme than modified immunogenicity. A modification may also change the performance of the enzyme, such as the wash performance, thermo stability, storage stability and increased catalytical activity of the enzyme.

5

The ability of an enzyme to catalyze the degradation of various naturally occurring substrates present on the objects to be cleaned during e.g. wash is often referred to as its washing ability, wash-ability, detergency, or wash performance.

10 Throughout this application the term wash performance will be used to encompass this property.

Commercial enzyme applications

15

Industrial applications

Another aspect of the invention is a composition comprising at least one protein (polypeptide) or enzyme of the invention. The composition may comprise other polypeptides, proteins or enzymes and/or ingredients normally used in personal care products, such as shampoo, soap bars, skin lotion, skin creme, hair dye, toothpaste, household articles, agro chemicals, personal care products, such as cleaning preparations e.g. for contact lenses, cosmetics, toiletries, oral and dermal pharmaceuticals, compositions used for treating textiles, compositions used for manufacturing food, e.g. baking, and feed etc.

Examples of said proteins(polypeptides)/enzymes include enzymes exhibiting protease, lipolytic enzyme, oxidoreductase, carbohydrase, transferase, such as transglutaminase, phytase and/or anti-microbial polypeptide activity. These enzymes may be present as conjugates with reduced activity.

The protein of the invention may furthermore typically be used in detergent composition. It may be included in the detergent composition in the form of a non-dusting granulate, a stabilized liquid, or a protected enzyme. Non-dusting granulates may be produced, e.g., as disclosed in US 4,106,991 and 4,661,452 (both to Novo Industri A/S) and may optionally be coated by methods known in the art. Examples of waxy coating materials are poly(ethylene oxide) products (polyethylene glycol, PEG) with mean molecular weights of 1000 to 20000; ethoxylated nonylphenols having from 16 to 50 ethylene oxide units; ethoxylated fatty alcohols in which the alcohol contains from 12 to 20 carbon atoms and in which there are 15 to 80 ethylene oxide units; fatty alcohols; fatty acids; and mono- and di- and triglycerides of fatty acids. Examples of film-forming coating materials suitable for application by fluid bed techniques are given in patent GB 1483591. Liquid enzyme preparations may, for instance, be stabilized by adding a polyol such as propylene glycol, a sugar or sugar alcohol, lactic acid or boric acid according to established methods. Other enzyme stabilizers are well known in the art. Protected enzymes may be prepared according to the method disclosed in EP 238,216.

The detergent composition may be in any convenient form, e.g. as powder, granules, paste or liquid. A liquid detergent may be aqueous, typically containing up to 70% water and 0-30% organic solvent, or non-aqueous.

The detergent composition comprises one or more surfactants, each of which may be anionic, nonionic, cationic, or zwitterionic. The detergent will usually contain 0-50% of anionic surfactant such as linear alkylbenzenesulfonate (LAS), alpha-olefinsulfonate (AOS), alkyl sulfate (fatty alcohol sulfate) (AS), alcohol ethoxysulfate (AEOS or AES), secondary alkanesulfonates (SAS), alpha-sulfo fatty acid methyl esters, alkyl- or alkenylsuccinic acid, or soap. It may also contain 0-40% of nonionic surfactant such as

alcohol ethoxylate (AEO or AE), carboxylated alcohol ethoxylates, nonylphenol ethoxylate, alkylpolyglycoside, alkyl dimethylamine - oxide, ethoxylated fatty acid monoethanolamide, fatty acid monoethanolamide, or polyhydroxy alkyl fatty acid amide (e.g. as described in WO 92/06154).

The detergent composition may additionally comprise one or more other enzymes, such as e.g. proteases, amylases, lipolytic enzymes, cutinases, cellulases, peroxidases, oxidases, and further anti-microbial polypeptides.

The detergent may contain 1-65% of a detergent builder or complexing agent such as zeolite, diphosphate, triphosphate, phosphonate, citrate, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTMPA), alkyl- or alkenylsuccinic acid, soluble silicates or layered silicates (e.g. SKS-6 from Hoechst). The detergent may also be unbuilt, i.e. essentially free of detergent builder.

The detergent may comprise one or more polymers. Examples are carboxymethylcellulose (CMC), poly(vinylpyrrolidone) (PVP), polyethyleneglycol (PEG), poly(vinyl alcohol) (PVA), polycarboxylates such as polyacrylates, maleic/acrylic acid copolymers and lauryl methacrylate/acrylic acid copolymers.

25

The detergent may contain a bleaching system which may comprise a H_2O_2 source such as perborate or percarbonate which may be combined with a peracid-forming bleach activator such as tetraacetythylenediamine (TAED) or nonanoyloxybenzenesulfonate (NOBS). Alternatively, the bleaching system may comprise peroxyacids of, e.g., the amide, imide, or sulfone type.

The detergent composition of the invention comprising the polypeptide of the invention may be stabilized using conventional

stabilizing agents, e.g. a polyol such as propylene glycol or glycerol, a sugar or sugar alcohol, lactic acid, boric acid, or a boric acid derivative such as, e.g., an aromatic borate ester, and the composition may be formulated as described in, e.g., WO
5 92/19709 and WO 92/19708.

The detergent may also contain other conventional detergent ingredients such as, e.g., fabric conditioners including clays, foam boosters, suds suppressors, anti-corrosion agents, soil-
10 suspending agents, anti-soil-redeposition agents, dyes, bactericides, optical brighteners, or perfume.

The pH (measured in aqueous solution at use concentration) will usually be neutral or alkaline, e.g. in the range of 7-11.

15

Dishwashing composition

Further, a modified enzyme according to the invention may also be used in dishwashing detergents.

20

Dishwashing detergent compositions comprise a surfactant which may be anionic, non-ionic, cationic, amphoteric or a mixture of these types. The detergent will contain 0-90% of non-ionic surfactant such as low- to non-foaming ethoxylated propoxylated
25 straight-chain alcohols.

The detergent composition may contain detergent builder salts of inorganic and/or organic types. The detergent builders may be subdivided into phosphorus-containing and non-phosphorus-
30 containing types. The detergent composition usually contains 1-90% of detergent builders.

Examples of phosphorus-containing inorganic alkaline detergent builders, when present, include the water-soluble salts espe-

cially alkali metal pyrophosphates, orthophosphates, and polyphosphates. An example of phosphorus-containing organic alkaline detergent builder, when present, includes the water-soluble salts of phosphonates. Examples of non-phosphorus-containing inorganic builders, when present, include water-soluble alkali metal carbonates, borates and silicates as well as the various types of water-insoluble crystalline or amorphous aluminosilicates of which zeolites are the best-known representatives.

10 Examples of suitable organic builders include the alkali metal, ammonium and substituted ammonium, citrates, succinates, malonates, fatty acid sulphonates, carboxymethoxy succinates, ammonium polyacetates, carboxylates, polycarboxylates, amino-polycarboxylates, polyacetyl carboxylates and polyhydroxysul-
15 phonates.

Other suitable organic builders include the higher molecular weight polymers and co-polymers known to have builder properties, for example appropriate polyacrylic acid, polymaleic and poly-
20 acrylic/polymaleic acid copolymers and their salts.

The dishwashing detergent composition may contain bleaching agents of the chlorine/bromine-type or the oxygen-type. Examples of inorganic chlorine/bromine-type bleaches are lithium, sodium
25 or calcium hypochlorite and hypobromite as well as chlorinated trisodium phosphate. Examples of organic chlorine/bromine-type bleaches are heterocyclic N-bromo and N-chloro imides such as trichloroisocyanuric, tribromoisocyanuric, dibromoisocyanuric and dichloroisocyanuric acids, and salts thereof with water-
30 solubilizing cations such as potassium and sodium. Hydantoin compounds are also suitable.

The oxygen bleaches are preferred, for example in the form of an inorganic persalt, preferably with a bleach precursor or as a

peroxy acid compound. Typical examples of suitable peroxy bleach compounds are alkali metal perborates, both tetrahydrates and monohydrates, alkali metal percarbonates, persilicates and perphosphates. Preferred activator materials are TAED and glycerol
5 triacetate.

The dishwashing detergent composition of the invention may be stabilized using conventional stabilizing agents for the enzyme(s), e.g. a polyol such as e.g. propylene glycol, a sugar or
10 a sugar alcohol, lactic acid, boric acid, or a boric acid derivative, e.g. an aromatic borate ester.

The dishwashing detergent composition of the invention may also contain other conventional detergent ingredients, e.g. defloc-
15 culant material, filler material, foam depressors, anti-corrosion agents, soil-suspending agents, sequestering agents, anti-soil redeposition agents, dehydrating agents, dyes, bactericides, fluorescers, thickeners and perfumes.

20 Finally, the enzyme of the invention may be used in conventional dishwashing-detergents, e.g. in any of the detergents described in any of the following patent publications:

EP 518719, EP 518720, EP 518721, EP 516553, EP 516554,
25 EP 516555, GB 2200132, DE 3741617, DE 3727911, DE 4212166,
DE 4137470, DE 3833047, WO 93/17089, DE 4205071, WO 52/09680, WO
93/18129, WO 93/04153, WO 92/06157, WO 92/08777, EP 429124, WO
93/21299, US 5141664, EP 561452, EP 561446, GB 2234980,
WO 93/03129, EP 481547, EP 530870, EP 533239, EP 554943,
30 EP 346137, US 5112518, EP 318204, EP 318279, EP 271155,
EP 271156, EP 346136, GB 2228945, CA 2006687, WO 93/25651,
EP 530635, EP 414197, US 5240632.

Personal care applications

A particularly useful application area for low allergenic proteins or of proteins with low cross-reactivity to environmental allergens would be in personal care products where the end-user is in close contact with the protein, and where certain problems with allergenicity has been encountered in experimental set-ups (Kelling et al., J. All. Clin. Imm., 1998, Vol. 101, pp. 179-187 and Johnston et al., Hum. Exp. Toxicol., 1999, Vol.18, p. 527).

10

First of all the conjugate or compositions of the invention can advantageously be used for personal care products, such as hair care and hair treatment products. This include products such as shampoo, balsam, hair conditioners, hair waving compositions, hair dyeing compositions, hair tonic, hair liquid, hair cream, shampoo, hair rinse, hair spray.

Further contemplated are oral care products such as dentifrice, oral washes, chewing gum.

Also contemplated are skin care products and cosmetics, such as skin cream, skin milk, cleansing cream, cleansing lotion, cleansing milk, cold cream, cream soap, nourishing essence, skin lotion, milky lotion, calamine lotion, hand cream, powder soap, transparent soap, sun oil, sun screen, shaving foam, shaving cream, baby oil lipstick, lip cream, creamy foundation, face powder, powder eye-shadow, powder, foundation, make-up base, essence powder, whitening powder.

30

Also for contact lenses hygiene products the conjugate of the invention can be used advantageously. Such products include cleaning and disinfection products for contact lenses.

Proteases

Proteases are well-known active ingredients for cleaning of contact lenses. They hydrolyse the proteinaceous soil on the lens and thereby makes it soluble. Removal of the protein soil is essential for the wearing comfort.

Proteases are also effective ingredients in skin cleaning products, where they remove the upper layer of dead keratinaceous skin cells and thereby make the skin look brighter and fresher.

Proteases are also used in oral care products, especially for cleaning of dentures, but also in dentifrices.

15

Further, proteases are used in toiletries, bath and shower products, including shampoos, conditioners, lotions, creams, soap bars, toilet soaps, and liquid soaps.

20 Lipolytic enzymes

Lipolytic enzymes can be applied for cosmetic use as active ingredients in skin cleaning products and anti-acne products for removal of excessive skin lipids, and in bath and shower products such as creams and lotions as active ingredients for skin care.

Lipolytic enzymes can also be used in hair cleaning products (e.g. shampoos) for effective removal of sebum and other fatty material from the surface of hair.

30

Lipolytic enzymes are also effective ingredients in products for cleaning of contact lenses, where they remove lipid deposits from the lens surface.

Oxidoreductases

5 The most common oxidoreductase for personal care purposes is an oxidase (usually glucose oxidase) with substrate (e.g. glucose) that ensures production of H_2O_2 , which then will initiate the oxidation of for instance SCN^- or I^- into antimicrobial reagents ($SCNO^-$ or I_2) by a peroxidase (usually lactoperoxidase). This enzymatic complex is known in nature from e.g. milk and saliva.

It is being utilised commercially as anti-microbial system in oral care products (mouth rinse, dentifrice, chewing gum) where it also can be combined with an amyloglucosidase to produce the
15 glucose. These systems are also known in cosmetic products for preservation.

Anti-microbial systems comprising the combination of an oxidase and a peroxidase are known in the cleaning of contact lenses.

20

Another application of oxidoreductases is oxidative hair dyeing using oxidases, peroxidases and laccases.

Free radicals formed on the surface of the skin (and hair) known
25 to be associated with the ageing process of the skin (spoilage of the hair). The free radicals activate chain reactions that lead to destruction of fatty membranes, collagen, and cells. The application of free radical scavengers such as Superoxide dismutase into cosmetics is well known (R. L. Goldemberg, DCI, Nov. 93, p.
30 48-52).

Protein disulfide isomerase (PDI) is also an oxidoreductase. It can be utilised for waving of hair (reduction and reoxidation of

disulfide bonds in hair) and repair of spoiled hair (where the damage is mainly reduction of existing disulfide bonds).

Carbohydrases

5

Plaque formed on the surface of teeth is composed mainly of polysaccharides. They stick to the surface of the teeth and the microorganisms. The polysaccharides are mainly α -1,6 bound glucose (dextran) and α -1,3 bound glucose (mutan). The application of different types of glucanases such as mutanase and dextranase helps hydrolysing the sticky matrix of plaque, making it easier to remove by mechanical action.

Also other kinds of biofilm for instance the biofilm formed in lens cases can be removed by the action of glucanases.

Food and Feed

Further conjugated enzymes or polypeptides with reduced immunogenicity according to the invention may advantageously be used in the manufacturing of food and feed.

Proteases

25

The gluten in wheat flour is the essential ingredient responsible for the ability of flour to be used in baked foodstuffs. Proteolytic enzymes are sometimes needed to modify the gluten phase of the dough, e.g. a hard wheat flour can be softened with a protease.

30

Neutrase® is a commercially available neutral metallo protease that can be used to ensure a uniform dough quality and bread texture, and to improve flavour. The gluten proteins are degraded

either moderately or more extensively to peptides, whereby close control is necessary in order to avoid excessive softening of the dough.

5 Proteases are also used for modifying milk protein.

To coagulate casein in milk when producing cheese proteases such as rennet or chymosin may be used.

10 In the brewery industry proteases are used for brewing with unmalted cereals and for controlling the nitrogen content.

In animal feed products proteases are used so to speak to expand the animals digestion system.

15

Lipolytic enzymes

Addition of lipolytic enzyme results in improved dough properties and an improved breadmaking quality in terms of larger volume,
20 improved crumb structure and whiter crumb colour. The observed effect can be explained by a mechanism where the lipolytic enzyme changes the interaction between gluten and some lipids fragment during dough mixing. This results in an improved gluten network.

25 The flavour development of blue roan cheese (e.g. Danablu), certain Italian type cheese, and other dairy products containing butter-fat, are dependent on the degradation of milk fat into free fatty acids. Lipolytic enzymes may be used for developing flavour in such products.

30

In the oil- and fat producing industry lipases are used e.g. to minimize the amount of undesirable side-products, to modify fats by interesterification, and to synthesis of esters.

Oxidoreductases

Further oxidoreductases with reduced immunogenicity according to the invention may advantageously be used in the manufacturing of
5 food and feed.

Several oxidoreductases are used for baking, glucose oxidase, lipoxygenase, peroxidase, catalase and combinations hereof. Traditionally, bakers strengthen gluten by adding ascorbic acid and
10 potassium bromate. Some oxidoreductases can be used to replace bromate in dough systems by oxidation of free sulfhydryl units in gluten proteins. Hereby disulphide linkages are formed resulting in stronger, more elastic doughs with greater resistance.

15 Gluzyme™ (Novozymes A/S) is a glucose oxidase preparation with catalase activity that can be used to replace bromate. The dough strengthen is measured as greater resistance to mechanical shock, better oven spring and larger loaf volume.

20 Carbohydrases

Flour has varying content of amylases leading to differences in the baking quality. Addition of amylases can be necessary in order to standardize the flour. Amylases and pentosanases generally
25 provide sugar for the yeast fermentation, improve the bread volume, retard retrogradation, and decrease the staling rate and stickiness that results from pentosan gums. Examples of carbohydrases are given below.

30 Certain maltogenic amylases can be used for prolonging the shelf life of bread for two or more days without causing gumminess in the product. Selectively modifies the gelatinized starch by cleaving from the non-reducing end of the starch molecules, low molecular weight sugars and dextrins. The starch is modified in

such a way that retrogradation is less likely to occur. The produced low-molecular-weight sugars improve the baked goods water retention capacity without creating the intermediate-length dex-
trins that result in gumminess in the finished product. The en-
zyme is inactivated during bread baking, so it can be considered
a processing aid that does not have to be declared on the label.
Overdosing of Novamyl can almost be excluded.

The bread volume can be improved by fungal α -amylases which fur-
ther provide good and uniform structure of the bread crumb. Said
 α -amylases are endoenzymes that produce maltose, dextrans and
glucose. Cereal and some bacterial α -amylases are inactivated at
temperatures above the gelatinization temperature of starch,
therefore when added to wheat dough it results in a low bread
volume and a sticky bread interior. Fungamyl has the advantage of
being thermolabile and is inactivated just below the gelatiniza-
tion temperature.

Enzyme preparations containing a number of pentosanase and hemi-
cellulase activities can improve the handling and stability of
the dough, and improves the freshness, the crumb structure and
the volume of the bread.

By hydrolysing the pentosans fraction in flour, it will lose a
great deal of its water-binding capacity, and the water will then
be available for starch and gluten. The gluten becomes more pli-
able and extensible, and the starch gelatinizes more easily. Pen-
tosanases can be used in combination with or as an alternative to
emulsifiers.

30

Further carbohydrases are used for producing syrups from starch,
which are widely used in soft drinks, sweets, meat products,
dairy products, bread products, ice cream, baby food, jam etc.

The conversion of starch is normally carried out three steps. First the starch is liquefied, by the use of α -amylases. Maltodextrins, primary consisting of oligosaccharides and dextrins, are obtained.

The mixture is then treated with an amyloglucosidase for hydrolysing the oligosaccharides and dextrins into glucose. This way a sweeter product is obtained. If high maltose syrups are desired β -amylases alone or in combination with a pullulanase (de-branching enzyme) may be used.

The glucose mixture can be made even sweeter by isomerization to fructose. For this an immobilized glucose isomerase can be used.

In the sugar industry, it is common practice to speed up the break down of present starch in cane juices. Thereby the starch content in the raw sugar is reduced and filtration at the refinery facilitated.

Furthermore dextranases are used to break down dextran in raw sugar juices and syrups.

In the alcohol industry α -amylases is advantageously being used for thinning of starch in distilling mashes.

In the brewing industry α -amylases is used for adjunct liquefaction.

In the dairy industry β -galactosidases (lactase) is used when producing low lactose milk for persons suffering from lactose malabsorption.

When flavoured milk drinks are produced from lactase-treated milk, the addition of sugar can be reduced without reducing the sweetness of the product.

- 5 In the production of condensed milk, lactose crystallization can be avoided by lactase treatment, and the risk of thickening caused by casein coagulation in lactose crystals is thus reduced.

When producing ice cream made from lactase-treated milk (or whey)
10 no lactose crystals will be formed and the defect, sandiness, will not occur.

Further, xylanases are known to be used within a number of food/feed industrial applications as described in WO 94/21785
15 (Novo Nordisk A/S).

α -amylases are used in the animal feed industry to be added to cereal-containing feed to improve the digestibility of starch.

20

Anti-microbial polypeptides

Certain bacteriolytic enzymes may be used e.g. to wash carcasses in the meat packing industry (see US patent no. 5,354,681 from
25 Novo Industri A/S)

Transferases

Transglutaminases with reduced immunogenicity according to the
30 invention may advantageously be used in the manufacturing of food and feed.

Transglutaminases has the ability to crosslinking protein.

This property can be used for gelling of aqueous phases containing proteins. This may be used for when producing of spreads (DK patent application no. 1071/84 from Novo Nordisk A/S).

- 5 Transglutaminases are being used for improvement of baking quality of flour e.g. by modifying wheat flour to be used in the preparation of cakes with improved properties, such as improved taste, dent, mouth-feel and a higher volume (see JP 1-110147).
- 10 Further producing paste type food material e.g. used as fat substitution in foods as ice cream, toppings, frozen desserts, mayonnaises and low fat spreads (see WO 93/22930 from Novo Nordisk A/S).
- 15 Furthermore for preparation of gels for yoghurt, mousses, cheese, puddings, orange juice, from milk and milk-like products, and binding of chopped meat product, improvement of taste and texture of food proteins (see WO 94/21120 and WO 94/21129 from Novo Nordisk A/S).

20

Phytases

Phytases of the invention may advantageously be used in the manufacturing of food, such as breakfast cereal, cake, sweets,
25 drinks, bread or soup etc., and animal feed.

Phytases may be used either for exploiting the phosphorus bound in the phytate/phytic acid present in vegetable protein sources
30 or for exploiting the nutritionally important minerals bound in phytic acid complexes.

Microbial phytase may be added to feedstuff of monogastric animals in order to avoid supplementing the feed with inorganic phosphorus (see US patent no. 3,297,548).

5 Further phytases may be used in soy processing. Soyabean meal may contain high levels of the anti-nutritional factor phytate which renders this protein source unsuitable for application in baby food and feed for fish, calves and other non-ruminants, since the phytate chelates essential minerals present therein (see EP 0 420
10 358).

Also for baking purposes phytases may be used. Bread with better quality can be prepared by baking divided pieces of a dough containing wheat flour etc. and phytase (see JP-0-3076529-A).

15

A high phytase activity as in koji mold are known to be used for producing refined sake (see JP-0-6070749-A).

Textile applications

20

Proteases

Proteases are used for degumming and sand washing of silk.

25 Lipolytic enzymes

Lipolytic enzymes are used for removing fatty matter containing hydrophobic esters (e.g. triglycerides) during the finishing of textiles (see e.g. WO 93/13256 from Novo Nordisk A/S).

30

Oxidoreductases

In bleach clean up of textiles catalases may serve to remove excess hydrogen peroxide.

Carbohydrases

Cellulolytic enzymes are widely used in the finishing of denim
5 garments in order to provide a localized variation in the colour
density of the fabric (Enzyme facilitated "stone wash").

Also cellulolytic enzymes find use in the bio-polishing process.
Bio-Polishing is a specific treatment of the yarn surface which
10 improves fabric quality with respect to handle and appearance
without loss of fabric wettability. Bio-polishing may be obtained
by applying the method described e.g. in WO 93/20278.

During the weaving of textiles, the threads are exposed to con-
15 siderable mechanical strain. In order to prevent breaking, the
threads are usually reinforced by the coating (sizing) with a ge-
latinous substance (size). The most common sizing agent is starch
in native or modified form. A uniform and durable finish can thus
be obtained only after removal of the size from the fabric, the
20 so-called desizing. Desizing of fabrics sized with a size con-
taining starch or modified starch is preferably facilitated by
use of amylolytic enzymes.

25 Oral and dermal pharmaceuticals

Proteases

Different combinations of highly purified proteases (e.g. Trypsin
30 and Chymotrypsin) are used in pharmaceuticals to be taken orally,
and dermal pharmaceuticals for combating e.g inflammations, ede-
mata and injuries.

Leather productionTransferase

- 5 Transglutaminase is known to be used to casein-finishing leather by acting as a hardening agent (see WO 94/13839 from Novo Nordisk).

Hard surface cleaning

10

Cleaning of hard surfaces e.g. in the food industry is often difficult, as equipment used for producing dairies, meat, sea food products, beverages etc. often have a complicated shape. The use of surfactant compositions in the form gels and foams comprising
15 enzymes have shown to facilitate and improve hard surface cleaning. Enzymes, which advantageously may be added in such surfactant compositions, are in particular proteases, lipolytic enzymes, amylases and cellulases.

- 20 Such hard surface cleaning compositions comprising enzymes may also advantageously be used in the transport sector, for instance for washing cars and for general vessel wash.

25

Furthermore this invention relates to the method by which the protein variants are being synthesised and expressed in host cells. This is achieved by culturing host cells capable of expressing a polypeptide in a suitable culture medium to obtain
30 expression and secretion of the polypeptide into the medium, followed by isolation of the polypeptide from the culture medium. The host cell may be any cell suitable for the large-scale production of proteins, capable of expressing a protein and being transformed by an expression vector.

The host cell comprises a DNA construct as defined above, optionally the cells may be transformed with an expression vector comprising a DNA construct as defined above. The host cell is selected from any suitable cell, such as a bacterial cell, a fungal cell, an animal cell, such as an insect cell or a mammalian cell, or a plant cell.

10

Immunotherapy

A number of vaccination approaches have been described to for infective diseases as well as for non-infective diseases (such as cancers). In a number of cases, the antigen provided is an isolated protein or protein-adjuvant mixture and more and more often, the protein is recombinant (e.g. the hepatitis B vaccine from Merck & Co). In these cases, it could be desirable to modify the immunogenicity of the antigen vaccine, such that it offers a stronger or more specific protection. This can be achieved by protein engineering of the amino acid sequence of the antigen, and would be greatly facilitated by the use of the methods of this invention for identification of epitopes on the antigen vaccine to be the favored sites for modification.

There are several examples of vaccine molecules that have been engineered to achieve a specific immune protection against virus, parasites or cancer (Ryu and Nam, Biotechnol. Prog., 2000, vol. 16 pp.2-16; and references cited therein). "The goal is often to vaccinate with a minimal structure consisting of a well-defined antigen, to stimulate an effective specific immune response, while avoiding potentially hazardous risks" (Ryu and Nam, Biotechnol. Prog., 2000, vol. 16 pp.2-16). Thus, the methods of this invention can be used to identify such minimal structures that define an antigen (or epitope thereof) whether

in the form of the parent protein scaffold with a number of mutations introduced in it, or whether it is in the form of the antibody binding peptides themselves.

5

Allergen vaccines

Today, a patient suffering allergic disease may be subjected to allergy vaccine therapy using allergens selected on the basis of
10 testing the specificity of the patient's serum IgE against a bank of allergen extracts (or similar specificity tests of the patient's sensitization such as skin prick test.

One could improve the quality of characterization by using anti-
15 body binding peptides corresponding to various epitope sequences on the protein allergens of interest. This would require a kit comprising reagents for such specificity characterization, e.g. the antibody binding peptides of desired specificity. It would be preferred to use antibody binding sequences in the kit, which
20 correspond to defined epitope sequences known to be specific for the allergen under investigation (i.e. not identified on other allergens and/or not cross-reacting with sera raised against other allergens). This kit would be useful to specifying which allergy the patient is suffering from. This kit will lead to a
25 more specific answer than those kits used today, and hence to a better selection of allergen vaccine therapy for the individual patient.

Further, the knowledge about cross-reacting epitopes may improve
30 vaccine development.

In an extension of this approach, one could also characterize the patient's serum by identifying the corresponding antibody binding peptides among a random display library using the afore-

mentioned methods. This again may lead to a better selection of allergen vaccine therapy.

Further, one could use the individual antibody binding sequences
5 as allergen vaccines leading to more specific allergen vaccine.
These antibody binding sequences could be administered in an
isolated form or fused to a membrane protein of the phage display system, or to another protein, which may have beneficial effect for the immunoprotective effect of the antibody binding
10 peptide (Dalum et al., Nature Biotechnology, 1999, Vol. 17, pp. 666-669).

15 D) Variations possible.

Parent protein

20

The "parent protein" can in principle be any protein molecule of biological origin, non-limiting examples of which are peptides, polypeptides, proteins, enzymes, post-translationally modified polypeptides such as lipopeptides or glycosylated peptides,
25 anti-microbial peptides or molecules, and proteins having pharmaceutical properties etc.

Accordingly the invention relates to a method, wherein the "parent protein" is chosen from the group consisting of polypeptides, small peptides, lipopeptides, antimicrobials, and pharmaceutical polypeptides.
30

The term "pharmaceutical polypeptides" is defined as polypeptides, including peptides, such as peptide hormones, proteins

and/or enzymes, being physiologically active when introduced into the circulatory system of the body of humans and/or animals.

Pharmaceutical polypeptides are potentially immunogenic as they
5 are introduced into the circulatory system.

Examples of "pharmaceutical polypeptides" contemplated according to the invention include insulin, ACTH, glucagon, somatostatin, somatotropin, thymosin, parathyroid hormone, pigmentary hormones,
10 somatomedin, erythropoietin, luteinizing hormone, chorionic gonadotropin, hypothalamic releasing factors, antidiuretic hormones, thyroid stimulating hormone, relaxin, interferon, thrombopoietin (TPO) and prolactin.

15 However, the proteins are preferably to be used in industry, housekeeping and/or medicine, such as proteins used in personal care products (for example shampoo; soap; skin, hand and face lotions; skin, hand and face cremes; hair dyes; toothpaste), food (for example in the baking industry), detergents and phar-
20 maceuticals.

Antimicrobial peptides.

The antimicrobial peptide (AMP) may be, e.g., a membrane-active
25 antimicrobial peptide, or an antimicrobial peptide affecting/interacting with intracellular targets, e.g. binding to cell DNA. The AMP is generally a relatively short peptide, consisting of less than 100 amino acid residues, typically 20-80 residues. The antimicrobial peptide has bactericidal and/or fungicidal ef-
30 fect, and it may also have antiviral or antitumour effects. It generally has low cytotoxicity against normal mammalian cells. The antimicrobial peptide is generally highly cationic and hydrophobic. It typically contains several arginine and lysine residues, and it may not contain a single glutamate or aspa-

ratate. It usually contains a large proportion of hydrophobic residues. The peptide generally has an amphiphilic structure, with one surface being highly positive and the other hydrophobic.

5 The bioactive peptide and the encoding nucleotide sequence may be derived from plants, invertebrates, insects, amphibians and mammals, or from microorganisms such as bacteria and fungi.

The antimicrobial peptide may act on cell membranes of target microorganisms, e.g. through nonspecific binding to the mem-
10 brane, usually in a membrane-parallel orientation, interacting only with one face of the bilayer.

The antimicrobial peptide typically has a structure belonging to one of five major classes: a helical, cystine-rich (defensin-like), b-sheet, peptides with an unusual composition of regular
15 amino acids, and peptides containing uncommon modified amino acids.

Examples of alpha-helical peptides are Magainin 1 and 2; Cecropin A, B and P1; CAP18; Andropin; Clavanin A or AK; Styelin D and C; and Buforin II. Examples of cystine-rich peptides are a-
20 Defensin HNP-1 (human neutrophil peptide) HNP-2 and HNP-3; b-Defensin-12, Drosomycin, g1-purothionin, and Insect defensin A. Examples of b-sheet peptides are Lactoferricin B, Tachyplesin I, and Protegrin PG1-5. Examples of peptides with an unusual composition are Indolicidin; PR-39; Bactenecin Bac5 and Bac7; and
25 Histatin 5. Examples of peptides with unusual amino acids are Nisin, Gramicidin A, and Alamethicin.

Another example is the antifungal peptide (AFP) from *Aspergillus giganteus*. As explained in detail in WO 94/01459, which is hereby incorporated by reference, the antifungal polypeptide
30 having the amino acid sequence shown in Fig. 1 has been found in several strains of the fungal species *A. giganteus*, an example of which is the *A. giganteus* strain deposited with the Centraalbureau voor Schimmelcultures (CBS) under the deposition number CBS 526.65.

However, the antifungal polypeptide, or variants thereof, suitable for the use according to the invention are expected to be derivable from other fungal species, especially other *Aspergillus* species such as *A. pallidus*, *A. clavatus*, *A. longivesica*, *A. rhizopodus* and *A. clavatonanicus*, because of the close relationship which exists between these species and *A. giganteus*.

In one embodiment of the invention the protein is an enzyme, such as glycosyl hydrolases, carbohydrases, peroxidases, proteases, lipolytic enzymes, phytases, polysaccharide lyases, oxidoreductases, transglutaminases and glycoisomerases, in particular the following.

Parent Proteases

Parent proteases (i.e. enzymes classified under the Enzyme Classification number E.C. 3.4 in accordance with the Recommendations (1992) of the International Union of Biochemistry and Molecular Biology (IUBMB)) include proteases within this group.

Examples include proteases selected from those classified under the Enzyme Classification (E.C.) numbers:

3.4.11 (i.e. so-called aminopeptidases), including 3.4.11.5 (Prolyl aminopeptidase), 3.4.11.9 (X-pro aminopeptidase), 3.4.11.10 (Bacterial leucyl aminopeptidase), 3.4.11.12 (Thermophilic aminopeptidase), 3.4.11.15 (Lysyl aminopeptidase), 3.4.11.17 (Tryptophanyl aminopeptidase), 3.4.11.18 (Methionyl aminopeptidase).

30

3.4.21 (i.e. so-called serine endopeptidases), including 3.4.21.1 (Chymotrypsin), 3.4.21.4 (Trypsin), 3.4.21.25 (Cucumisin), 3.4.21.32 (Brachyurin), 3.4.21.48 (Cerevisin) and 3.4.21.62 (Subtilisin);

3.4.22 (i.e. so-called cysteine endopeptidases), including
3.4.22.2 (Papain), 3.4.22.3 (Ficain), 3.4.22.6 (Chymopapain),
3.4.22.7 (Asclepain), 3.4.22.14 (Actinidain), 3.4.22.30 (Cari-
5 cain) and 3.4.22.31 (Ananain);

3.4.23 (i.e. so-called aspartic endopeptidases), including
3.4.23.1 (Pepsin A), 3.4.23.18 (Aspergillopepsin I), 3.4.23.20
(Penicillopepsin) and 3.4.23.25 (Saccharopepsin); and

10

3.4.24 (i.e. so-called metalloendopeptidases), including
3.4.24.28 (Bacillolysin).

Serine proteases

15 A serine protease is an enzyme which catalyzes the hydrolysis of
peptide bonds, and in which there is an essential serine residue
at the active site (White, Handler and Smith, 1973 "*Principles
of Biochemistry*," Fifth Edition, McGraw-Hill Book Company, NY,
pp. 271-272).

20

The bacterial serine proteases have molecular weights in the
20,000 to 45,000 Dalton range. They are inhibited by diisopro-
pylfluorophosphate. They hydrolyze simple terminal esters and
are similar in activity to eukaryotic chymotrypsin, also a
25 serine protease. A more narrow term, alkaline protease, covering
a sub-group, reflects the high pH optimum of some of the serine
proteases, from pH 9.0 to 11.0 (for review, see Priest (1977)
Bacteriological Rev. 41 711-753).

30 Subtilases

A sub-group of the serine proteases tentatively designated
subtilases has been proposed by Siezen et al., *Protein Engng.* 4
(1991) 719-737 and Siezen et al. *Protein Science* 6 (1997) 501-
523. They are defined by homology analysis of more than 170

amino acid sequences of serine proteases previously referred to as subtilisin-like proteases. A subtilisin was previously often defined as a serine protease produced by Gram-positive bacteria or fungi, and according to Siezen et al. now is a subgroup of the subtilases. A wide variety of subtilases have been identified, and the amino acid sequence of a number of subtilases has been determined. For a more detailed description of such subtilases and their amino acid sequences reference is made to Siezen et al. (1997).

10

Savinase-like subtilisin

One subgroup of the subtilases may be classified as savinase-like subtilisins, having at least 81% homology to Savinase, preferably at least 85% homology, more preferably at least 90% homology, even more preferably at least 96% homology, most preferably at least 98% homology to Savinase.

Parent subtilase

The term "parent subtilase" describes a subtilase defined according to Siezen et al. (1991 and 1997). For further details see description of "SUBTILASES" immediately above. A parent subtilase may also be a subtilase isolated from a natural source, wherein subsequent modifications have been made while retaining the characteristic of a subtilase. Furthermore, a parent subtilase may also be a subtilase which has been prepared by the DNA shuffling technique, such as described by J.E. Ness et al., Nature Biotechnology, 17, 893-896 (1999).

Alternatively the term "parent subtilase" may be termed "wild type subtilase".

Modification(s) of a subtilase variant

The term "modification(s)" used herein is defined to include chemical modification of a subtilase as well as genetic

manipulation of the DNA encoding a subtilase. The modification(s) can be replacement(s) of the amino acid side chain(s), substitution(s), deletion(s) and/or insertions in or at the amino acid(s) of interest.

5

Subtilase variant

In the context of this invention, the term subtilase variant or mutated subtilase means a subtilase that has been produced by an organism which is expressing a mutant gene derived from a parent
10 microorganism which possessed an original or parent gene and which produced a corresponding parent enzyme, the parent gene having been mutated in order to produce the mutant gene from which said mutated subtilase protease is produced when expressed in a suitable host.

15

Examples of relevant subtilisins comprise subtilisin BPN', subtilisin amylosacchariticus, subtilisin 168, subtilisin mesentericopeptidase, subtilisin Carlsberg, subtilisin DY, subtilisin 309,
20 subtilisin 147, PD498 (WO 93/24623), thermitase, aqualysin, Bacillus PB92 protease, proteinase K, Protease TW7, and Protease TW3.

Preferred commercially available protease enzymes include
25 Alcalase™, Savinase™, Primase™, Duralase™, Neutrase®, Dyrazym®, Esperase™, Pyrase®, Pancreatic Trypsin NOVO (PTN), Bio-Feed™ Pro, Clear-Lens Pro, and Relase® (Novozymes A/S), Maxatase™, Maxacal™, Maxapem™, Properase™, Purafect™, Purafect OxP™, (Genencor International Inc.).

30

It is to be understood that also protease variants are contemplated as the parent protease. Examples of such protease variants

are disclosed in EP 130.756 (Genentech), EP 214.435 (Henkel), WO 87/04461 (Amgen), WO 87/05050 (Genex), EP 251.446 (Genencor), EP 260.105 (Genencor), Thomas et al., (1985), Nature. 318, p. 375-376, Thomas et al., (1987), J. Mol. Biol., 193, pp. 803-813, Russell et al., (1987), Nature, 328, p. 496-500, WO 88/08028 (Genex),
5 WO 88/08033 (Amgen), WO 89/06279 (Novo Nordisk A/S), WO 91/00345 (Novo Nordisk A/S), EP 525 610 (Solvay) and WO 94/02618 (Gist-Brocades N.V.).

10 The activity of proteases can be determined as described in "Methods of Enzymatic Analysis", third edition, 1984, Verlag Chemie, Weinheim, vol. 5.

15 Parent Lipolytic enzymes

Lipolytic enzymes are classified in EC 3.1.1 Carboxylic Ester Hydrolases according to Enzyme Nomenclature (available at <http://www.chem.qmw.ac.uk/iubmb/enzyme>). The lipolytic enzyme may have a substrate specificity with an activity such as EC
20 3.1.1.3 triacylglycerol lipase, EC 3.1.1.4 phospholipase A2, EC 3.1.1.5 lysophospholipase, EC 3.1.1.26 galactolipase, EC 3.1.1.32 phospholipase A1, EC 3.1.1.73 feruloyl esterase or EC 3.1.1.74 cutinase.

25 The parent lipolytic enzyme may be prokaryotic, particularly a bacterial enzyme, e.g. from *Pseudomonas*. Examples are *Pseudomonas* lipases, e.g. from *P. cepacia* (US 5,290,694, pdb file 1OIL), *P. glumae* (N Frenken et al. (1992), Appl. Envir. Microbiol. 58 3787-3791, pdb files 1TAH and 1QGE), *P. pseudoalcaligenes* (EP
30 334 462) and *Pseudomonas* sp. strain SD 705 (FERM BP-4772) (WO 95/06720, EP 721 981, WO 96/27002, EP 812 910). The *P. glumae* lipase sequence is identical to the amino acid sequence of *Chromobacterium viscosum* (DE 3908131 A1). Other examples are bacte-

rial cutinases, e.g. from *Pseudomonas* such as *P. mendocina* (US 5,389,536) or *P. putida* (WO 88/09367).

Alternatively, the parent lipolytic enzyme may be eukaryotic, e.g. a fungal lipolytic enzyme such as lipolytic enzymes of the *Humicola* family and the *Zygomycetes* family and fungal cutinases.

Examples of fungal cutinases are the cutinases of *Fusarium solani pisi* (S. Longhi et al., *Journal of Molecular Biology*, 268 (4), 779-799 (1997)) and *Humicola insolens* (US 5,827,719).

The parent lipolytic enzyme may be fungal and may have an amino acid sequence that can be aligned with SEQ ID NO: 1 which is the amino acid sequence shown in positions 1-269 of SEQ ID NO: 2 of US 5,869,438 for the lipase from *Thermomyces lanuginosus* (synonym *Humicola lanuginosa*), described in EP 258 068 and EP 305 216 (trade name Lipolase). The parent lipolytic enzyme may particularly have an amino acid sequence with at least 50 % homology with SEQ ID NO: 1. In addition to the lipase from *T. lanuginosus*, other examples are a lipase from *Penicillium camembertii* (P25234), a lipase from *Fusarium*, lipase/phospholipase from *Fusarium oxysporum* (EP 130064, WO 98/26057), lipase from *F. heterosporum* (R87979), lysophospholipase from *Aspergillus foetidus* (W33009), phospholipase A1 from *A. oryzae* (JP-A 10-155493), lipase from *A. oryzae* (D85895), lipase/ferulic acid esterase from *A. niger* (Y09330), lipase/ferulic acid esterase from *A. tubingensis* (Y09331), lipase from *A. tubingensis* (WO 98/45453), lysophospholipase from *A. niger* (WO 98/31790), lipase from *F. solanii* having an isoelectric point of 6.9 and an apparent molecular weight of 30 kDa (WO 96/18729).

Other examples are the *Zygomycetes* family of lipases comprising lipases having at least 50 % homology with the lipase of *Rhizomucor miehei* (P19515). This family also includes the lipases

from *Absidia reflexa*, *A. sporophora*, *A. corymbifera*, *A. blakesleeana*, *A. griseola* (all described in WO 96/13578 and WO 97/27276) and *Rhizopus oryzae* (P21811). Numbers in parentheses indicate publication or accession to the EMBL, GenBank, GeneSeqp
5 or Swiss-Prot databases.

Examples of lipases include lipases derived from the following microorganisms. The indicated patent publications are in-
10 corporated herein by reference:

Humicola, e.g. *H. brevispora*, *H. brevis* var. *thermoidea*.

Pseudomonas, e.g. *Ps. fragi*, *Ps. stutzeri*, *Ps. cepacia* and *Ps. fluorescens* (WO 89/04361), or *Ps. plantarii* or *Ps. gladioli* (US patent no. 4,950,417 (Solvay enzymes)) or *Ps. alcaligenes*
15 and *Ps. pseudoalcaligenes* (EP 218 272) or.

Candida, e.g. *C. cylindracea* (also called *C. rugosa*) or *C. antarctica* (WO 88/02775) or *C. antarctica* lipase A or B (WO
20 94/01541 and WO 89/02916).

Geotricum, e.g. *G. candidum* (Schimada et al., (1989), J. Biochem., 106, 383-388).

Rhizopus, e.g. *R. delemar* (Hass et al., (1991), Gene 109, 107-113) or *R. niveus* (Kugimiya et al., (1992) Biosci.
25 Biotech. Biochem 56, 716-719) or *R. oryzae*.

Bacillus, e.g. *B. subtilis* (Dartois et al., (1993)

Biochemica et Biophysica acta 1131, 253-260) or

B. stearothermophilus (JP 64/7744992) or *B. pumilus* (WO
91/16422).

30

Specific examples of readily available commercial lipases include Lipolase® (WO 98/35026) Lipolase™ Ultra, Lipozyme®, Palatase®, Novozym® 435, Lecitase® (all available from Novozymes A/S).

Examples of other lipases are Lumafast™, *Ps. mendocian* lipase from Genencor Int. Inc.; Lipomax™, *Ps. pseudoalcaligenes* lipase from Gist Brocades/Genencor Int. Inc.; *Fusarium solani* lipase (cutinase) from Unilever; *Bacillus* sp. lipase from Solvay enzymes. Other lipases are available from other companies.

It is to be understood that also lipase variants are contemplated as the parent enzyme. Examples of such are described in e.g. WO 93/01285 and WO 95/22615.

10

The activity of the lipase can be determined as described in "Methods of Enzymatic Analysis", Third Edition, 1984, Verlag Chemie, Weinheim, vol. 4, or as described in AF 95/5 GB (available on request from Novozymes A/S).

15

Parent Oxidoreductases

Parent oxidoreductases (i.e. enzymes classified under the Enzyme Classification number E.C. 1 (Oxidoreductases) in accordance with the Recommendations (1992) of the International Union of Biochemistry and Molecular Biology (IUBMB)) include oxidoreductases within this group.

Examples include oxidoreductases selected from those classified under the Enzyme Classification (E.C.) numbers:

Glycerol-3-phosphate dehydrogenase NAD⁺ (1.1.1.8), Glycerol-3-phosphate dehydrogenase NAD(P)⁺ (1.1.1.94), Glycerol-3-phosphate 1-dehydrogenase NADP (1.1.1.94), Glucose oxidase (1.1.3.4), Hexose oxidase (1.1.3.5), Catechol oxidase (1.1.3.14), Bilirubin oxidase (1.3.3.5), Alanine dehydrogenase (1.4.1.1), Glutamate dehydrogenase (1.4.1.2), Glutamate dehydrogenase NAD(P)⁺ (1.4.1.3), Glutamate dehydrogenase NADP⁺ (1.4.1.4), L-Amino acid dehydrogenase (1.4.1.5), Serine dehydrogenase

(1.4.1.7), Valine dehydrogenase NADP^+ (1.4.1.8), Leucine dehydrogenase (1.4.1.9), Glycine dehydrogenase (1.4.1.10), L-Amino-acid oxidase (1.4.3.2.), D-Amino-acid oxidase (1.4.3.3), L-Glutamate oxidase (1.4.3.11), Protein-lysine 6-oxidase
5 (1.4.3.13), L-lysine oxidase (1.4.3.14), L-Aspartate oxidase (1.4.3.16), D-amino-acid dehydrogenase (1.4.99.1), Protein disulfide reductase (1.6.4.4), Thioredoxin reductase (1.6.4.5), Protein disulfide reductase (glutathione) (1.8.4.2), Laccase (1.10.3.2), Catalase (1.11.1.6), Peroxidase (1.11.1.7), Lipoxy-
10 genase (1.13.11.12), Superoxide dismutase (1.15.1.1)

Said Glucose oxidases may be derived from *Aspergillus niger*.

Said Laccases may be derived from *Polyporus pinsitus*, *My-*
15 *celiophthora thermophila*, *Coprinus cinereus*, *Rhizoctonia solani*, *Rhizoctonia praticola*, *Scytalidium thermophilum* and *Rhus vernicifera*. Because of the homology found between the above mentioned laccases (see WO 98/38287), they are considered to belong to the same class of laccases, namely the class of "Coprinus-like
20 laccases". Accordingly, in the present context, the term "Coprinus-like laccase" is intended to indicate a laccase which, on the amino acid level, displays a homology of at least 50% and less than 100% to the *Coprinus cinereus* laccase SEQ ID NO 3, or at least 55% and less than 100% to the *Coprinus cinereus* laccase SEQ
25 ID NO 3, or at least 60% and less than 100% to the *Coprinus cinereus* laccase SEQ ID NO 3, or at least 65% and less than 100% to the *Coprinus cinereus* laccase SEQ ID NO 3, or at least 70% and less than 100% to the *Coprinus cinereus* laccase SEQ ID NO 3, or at least 75% and less than 100% to the *Coprinus cinereus* laccase
30 SEQ ID NO 3, or at least 80% and less than 100% to the *Coprinus cinereus* laccase SEQ ID NO 3, or at least 85% and less than 100% to the *Coprinus cinereus* laccase SEQ ID NO 3, or at least 90% and less than 100% to the *Coprinus cinereus* laccase SEQ ID NO 3, at

least 95% and less than 100% or at least 98% and less than 100% to the *Coprinus cinereus* laccase SEQ ID NO 3.

Bilirubin oxidases may be derived from *Myrothecium verrucaria*.

5

The Peroxidase may be derived from e.g. Soy bean, Horseradish or *Coprinus cinereus*.

The Protein Disulfide reductase may be any of the mentioned in DK 10 patent applications No. 768/93, 265/94 and 264/94 (Novo Nordisk A/S), which are hereby incorporated as references, including Protein Disulfide reductases of bovine origin, Protein Disulfide reductases derived from *Aspergillus oryzae* or *Aspergillus niger*, and DsbA or DsbC derived from *Escherichia coli*.

15

Specific examples of readily available commercial oxidoreductases include Gluzyme™ (enzyme available from Novozymes A/S). However, other oxidoreductases are available from others.

It is to be understood that also variants of oxidoreductases are 20 contemplated as the parent enzyme.

The activity of oxidoreductases can be determined as described in "Methods of Enzymatic Analysis", third edition, 1984, Verlag Chemie, Weinheim, vol. 3.

25

Parent Carbohydrases

Parent carbohydrases may be defined as all enzymes capable of 30 breaking down carbohydrate chains (e.g. starches) of especially five and six member ring structures (i.e. enzymes classified under the Enzyme Classification number E.C. 3.2 (glycosidases) in accordance with the Recommendations (1992) of the International Union of Biochemistry and Molecular Biology (IUBMB)). Also in-

cluded in the group of carbohydrases according to the invention are enzymes capable of isomerizing carbohydrates e.g. six member ring structures, such as D-glucose to e.g. five member ring structures like D-fructose.

5

Examples include carbohydrases selected from those classified under the Enzyme Classification (E.C.) numbers:

α -amylase (3.2.1.1) β -amylase (3.2.1.2), glucan 1,4- α -
10 glucosidase (3.2.1.3), cellulase (3.2.1.4), endo-1,3(4)- β -
glucanase (3.2.1.6), endo-1,4- β -xylanase (3.2.1.8), dextranase
(3.2.1.11), chitinase (3.2.1.14), polygalacturonase (3.2.1.15),
lysozyme (3.2.1.17), β -glucosidase (3.2.1.21), α -galactosidase
(3.2.1.22), β -galactosidase (3.2.1.23), amylo-1,6-glucosidase
15 (3.2.1.33), xylan 1,4- β -xylosidase (3.2.1.37), glucan endo-1,3- β -
D-glucosidase (3.2.1.39), α -dextrin endo-1,6-glucosidase
(3.2.1.41), sucrose α -glucosidase (3.2.1.48), glucan endo-1,3- α -
glucosidase (3.2.1.59), glucan 1,4- β -glucosidase (3.2.1.74), glu-
can endo-1,6- β -glucosidase (3.2.1.75), arabinan endo-1,5- α -
20 arabinosidase (3.2.1.99), lactase (3.2.1.108), chitonanase
(3.2.1.132) and xylose isomerase (5.3.1.5).

Examples of relevant carbohydrases include α -1,3-glucanases de-
rived from *Trichoderma harzianum*; α -1,6-glucanases derived from a
25 strain of *Paecilomyces*; β -glucanases derived from *Bacillus sub-*
tilis; β -glucanases derived from *Humicola insolens*; β -glucan-ases
derived from *Aspergillus niger*; β -glucanases derived from a
strain of *Trichoderma*; β -glucanases derived from a strain of
Oerskovia xanthineolytica; exo-1,4- α -D-glucosidases (glucoamy-
30 lases) derived from *Aspergillus niger*; α -amylases derived from
Bacillus subtilis; α -amylases derived from *Bacillus amyloliquefa-*

ciens; α -amylases derived from *Bacillus stearothermophilus*; α -amylases derived from *Aspergillus oryzae*; α -amylases derived from non-pathogenic microorganisms; α -galactosidases derived from *Aspergillus niger*; Pentosanases, xylanases, cellobiases, cellulases, hemi-cellulases derived from *Hemicella insolens*; cellulases derived from *Trichoderma reesei*; cellulases derived from non-pathogenic mold; pectinases, cellulases, arabinases, hemi-celluloses derived from *Aspergillus niger*; dextranases derived from *Penicillium lilacinum*; endo-glucanase derived from non-pathogenic mold; pullulanases derived from *Bacillus acidopulliticus*; β -galactosidases derived from *Kluyveromyces fragilis*; xylanases derived from *Trichoderma reesei*;

Specific examples of readily available commercial carbohydrases include Alpha-GalTM, Bio-FeedTM Alpha, Bio-FeedTM Beta, Bio-FeedTM Plus, Bio-FeedTM Plus, Novozyme[®] 188, Carezyme[®] (SEQ ID NO. 5), Celluclast[®], Cellusoft[®], Ceremyl[®], CitrozymTM, DenimaxTM, DezymeTM, DextrozymeTM, Finizym[®], FungamylTM, GamanaseTM, Glucanex[®], Lactozym[®], MaltogenaseTM, PentopanTM, PectinexTM, Promozyme[®], PulpzymeTM, NovamylTM, Termamyl[®], AMG (Amyloglucosidase Novo), Maltogenase[®], Sweetzyme[®], Aquazym[®], Natalase[®] (SEQ ID NO. 4), SP722, AA560 (all enzymes available from Novozymes A/S). Other carbohydrases are available from other companies.

The parent cellulase is preferably a microbial cellulase. As such, the cellulase may be selected from bacterial cellulases, e.g. *Pseudomonas* cellulases or *Bacillus*, such as the *Bacillus* strains described in US 4,822,516, US 5,045,464 or EP 468 464, or *B. lautus* (cf. WO 91/10732), cellulases. More preferably, the parent cellulases may be a fungal cellulase, in particular *Hemicella*, *Trichoderma*, *Irpex*, *Aspergillus*, *Penicillium*, *Myceliophthora* or *Fusarium* cellulases. Examples of suitable parent

cellulases are described in, e.g. WO 91/17244. Examples of suitable *Trichoderma* cellulases are those described in T.T. Teeri, Gene 51, 1987, pp. 43-52. Preferably, the parent cellulase is selected from the cellulases classified in family 45, e.g. the enzymes EG B (*Pseudomonas fluorescens*) and EG V (*Humicola insolens*), as described in Henrissat, B. et al.: Biochem. J. (1993), 293, p. 781-788.

10 The Termamyl-like α -amylase

It is well known that a number of α -amylases produced by *Bacillus* spp. are highly homologous on the amino acid level. For instance, the *B. licheniformis* α -amylase comprising the amino acid sequence shown in SEQ ID NO: 4 of WO 00/29560 (commercially available as Termamyl™) has been found to be about 89% homologous with the *B. amyloliquefaciens* α -amylase comprising the amino acid sequence shown in SEQ ID NO: 5 of WO 00/29560 and about 79% homologous with the *B. stearothermophilus* α -amylase comprising the amino acid sequence shown in SEQ ID NO: 3 of WO 00/29560. Further homologous α -amylases include an α -amylase derived from a strain of the *Bacillus* sp. NCIB 12289, NCIB 12512, NCIB 12513 or DSM 9375, all of which are described in detail in WO 95/26397, and the α -amylase described by Tsukamoto et al., Biochemical and Biophysical Research Communications, 151 (1988), pp. 25-31.

25

Still further homologous α -amylases include the α -amylase produced by the *B. licheniformis* strain described in EP 0252666 (ATCC 27811), and the α -amylases identified in WO 91/00353 and WO 94/18314. Other commercial Termamyl-like *B. licheniformis* α -amylases are Optitherm™ and Takatherm™ (available from Solvay), Maxamyl™ (available from Gist-brocades/Genencor), Spezym AA™ and Spezyme Delta AA™ (available from Genencor), and Keistase™

30

(available from Daiwa).

Because of the substantial homology found between these α -amylases, they are considered to belong to the same class of α -amylases, namely the class of "Termamyl-like α -amylases".

Accordingly, in the present context, the term "Termamyl-like α -amylase" is intended to indicate an α -amylase which, at the amino acid level, exhibits a substantial homology to Termamyl™, i.e., the *B. licheniformis* α -amylase having the amino acid sequence shown in SEQ ID NO: 4 (WO 00/29560). In other words, a Termamyl-like α -amylase is an α -amylase which has the amino acid sequence shown in SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7 or 8 of WO 00/29560, and the amino acid sequence shown in SEQ ID NO: 1 of WO 95/26397 (the same as the amino acid sequence shown as SEQ ID NO: 7 of WO 00/29560) or in SEQ ID NO: 2 of WO 95/26397 (the same as the amino acid sequence shown as SEQ ID NO: 8 of 00/29560) or in Tsukamoto et al., 1988, (which amino acid sequence is shown in SEQ ID NO: 6 of WO 00/29560) or i) which displays at least 60% homology (identity), preferred at least 70%, more preferred at least 75%, even more preferred at least 80%, especially at least 85%, especially preferred at least 90%, especially at least 95%, even especially more preferred at least 97%, especially at least 99% homology with at least one of said amino acid sequences shown in SEQ ID NOS 1: or 2 or 3 or 4 or 5 or 6 or 7 or 8 of WO 00/29560 and/or ii) displays immunological cross-reactivity with an antibody raised against one or more of said α -amylases, and/or iii) is encoded by a DNA sequence which hybridizes, under the low to very high stringency conditions (said conditions described below) to the DNA sequences encoding the above-specified α -amylases which are apparent from SEQ ID NOS: 9, 10, 11, 12, and 32, respectively, of the present application (which encodes the amino acid sequences shown in SEQ ID NOS: 1, 2, 3, 4, and 5

herein, respectively), from SEQ ID NO: 4 of WO 95/26397 (which DNA sequence, together with the stop codon TAA, is shown in SEQ ID NO: 13 herein and encodes the amino acid sequence shown in SEQ ID NO: 8 herein) and from SEQ ID NO: 5 of WO 95/26397 (shown in
5 SEQ ID NO: 14 herein), respectively.

In connection with property i), the "homology" (identity) may be determined by use of any conventional algorithm, preferably by use of the gap programme from the GCG package version 8 (August
10 1994) using default values for gap penalties, i.e., a gap creation penalty of 3.0 and gap extension penalty of 0.1 (Genetic Computer Group (1991) Programme Manual for the GCG Package, version 8, 575 Science Drive, Madison, Wisconsin, USA 53711).

15 The parent Termamyl-like α -amylase backbone may in an embodiment have an amino acid sequence which has a degree of identity to SEQ ID NO: 4 (WO 00/29560) of at least 65%, preferably at least 70%, preferably at least 75%, more preferably at least 80%, more preferably at least 85%, even more preferably at least about 90%,
20 even more preferably at least 95%, even more preferably at least 97%, and even more preferably at least 99% identity determined as described above

A structural alignment between Termamyl[®] (SEQ ID NO: 4) and a
25 Termamyl-like α -amylase may be used to identify equivalent/corresponding positions in other Termamyl-like α -amylases. One method of obtaining said structural alignment is to use the Pile Up programme from the GCG package using default values of gap penalties, i.e., a gap creation penalty of 3.0 and
30 gap extension penalty of 0.1. Other structural alignment methods include the hydrophobic cluster analysis (Gaboriaud et al., (1987), FEBS LETTERS 224, pp. 149-155) and reverse threading (Huber, T ; Torda, AE, PROTEIN SCIENCE Vol. 7, No. 1 pp. 142-149

(1998).

Parent Glucoamylases

Parent glucoamylase contemplated according to the present
5 invention include fungal glucoamylases, in particular fungal
glucoamylases obtainable from an *Aspergillus* strain, such as an
Aspergillus niger or *Aspergillus awamori* glucoamylases and
variants or mutants thereof, homologous glucoamylases, and
further glucoamylases being structurally and/or functionally
10 similar to SEQ ID NO: 2 (WO 00/04136). Specifically contemplated
are the *Aspergillus niger* glucoamylases G1 and G2 disclosed in
Boel et al. (1984), "Glucoamylases G1 and G2 from *Aspergillus*
niger are synthesized from two different but closely related
mRNAs", EMBO J. 3 (5), p. 1097-1102,. The G2 glucoamylase is
15 disclosed in SEQ ID NO: 2 (WO 00/04136). The G1 glucoamylase is
disclosed in SEQ ID NO: 13 (WO 00/04136). Another AMG backbone
contemplated is *Talaromyces emersonii*, especially *Talaromyces*
emersonii DSM disclosed in WO 99/28448 (Novo Nordisk).

20 The homology referred to above of the parent glucoamylase is
determined as the degree of identity between two protein
sequences indicating a derivation of the first sequence from the
second. The homology may suitably be determined by means of
computer programs known in the art such as GAP provided in the
25 GCG program package (Program Manual for the Wisconsin Package,
Version 8, August 1994, Genetics Computer Group, 575 Science
Drive, Madison, Wisconsin, USA 53711) (Needleman, S.B. and
Wunsch, C.D., (1970), Journal of Molecular Biology, 48, p. 443-
453). Using Gap with the following settings for polypeptide
30 sequence comparison: Gap creation penalty of 3.0 and Gap
extension penalty of 0.1, the mature part of a polypeptide
encoded by an analogous DNA sequence of the invention exhibits a
degree of identity preferably of at least 60%, such as 70%, at
least 80%, at least 90%, more preferably at least 95%, more
35 preferably at least 97%, and most preferably at least 99% with

the mature part of the amino acid sequence shown in SEQ ID NO: 2 (WO 00/04136).

Preferably, the parent glucoamylase comprise the amino acid sequences of SEQ ID NO: 2 (WO 00/04136); or allelic variants thereof; or fragments thereof that has glucoamylase activity.

A fragment of SEQ ID NO: 2 is a polypeptide which have one or more amino acids deleted from the amino and/or carboxyl terminus of this amino acid sequence. For instance, the AMG G2 (SEQ ID NO: 2) is a fragment of the *Aspergillus niger* G1 glucoamylase (Boel et al. (1984), EMBO J. 3 (5), p. 1097-1102) having glucoamylase activity. An allelic variant denotes any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequences. An allelic variant of a polypeptide is a polypeptide encoded by an allelic variant of a gene.

It is to be understood that also carbohydrase variants are contemplated as the parent enzyme.

The activity of carbohydrases can be determined as described in "Methods of Enzymatic Analysis", third edition, 1984, Verlag Chemie, Weinheim, vol. 4.

Parent Transferases

Parent transferases (i.e. enzymes classified under the Enzyme Classification number E.C. 2 in accordance with the Recommendations (1992) of the International Union of Biochemistry and Molecular Biology (IUBMB)) include transferases within this group.

The parent transferases may be any transferase in the subgroups of transferases: transferases transferring one-carbon groups (E.C. 2.1); transferases transferring aldehyde or residues (E.C. 2.2); acyltransferases (E.C. 2.3); glucosyltransferases (E.C. 2.4); transferases transferring alkyl or aryl groups, other than methyl groups (E.C. 2.5); transferases transferring nitrogenous groups (2.6).

In a preferred embodiment the parent transferase is a transglutaminase E.C. 2.3.2.13 (Protein-glutamine μ -glutamyltransferase).

Transglutaminases are enzymes capable of catalyzing an acyl transfer reaction in which a gamma-carboxyamide group of a peptide-bound glutamine residue is the acyl donor. Primary amino groups in a variety of compounds may function as acyl acceptors with the subsequent formation of monosubstituted gamma-amides of peptide-bound glutamic acid. When the epsilon-amino group of a lysine residue in a peptide-chain serves as the acyl acceptor, the transferases form intramolecular or intermolecular gamma-glutamyl-epsilon-lysyl crosslinks.

Examples of transglutaminases are described in the pending DK patent application no. 990/94 (Novo Nordisk A/S).

The parent transglutaminase may be of human, animal (e.g. bovine) or microbial origin.

Examples of such parent transglutaminases are animal derived Transglutaminase, FXIIIa; microbial transglutaminases derived from *Physarum polycephalum* (Klein et al., Journal of Bacteriology, Vol. 174, p. 2599-2605); transglutaminases derived from *Streptomyces* sp., including *Streptomyces lavendulae*, *Streptomyces lydicus* (former *Streptomyces libani*) and *Streptoverticillium* sp., including *Streptoverticillium mobaraense*, *Streptoverticillium cinnamomeum*, and *Streptoverticillium griseocarneum* (Motoki et

al., US 5,156,956; Andou et al., US 5,252,469; Kaempfer et al., Journal of General Microbiology, Vol. 137, p. 1831-1892; Ochi et al., International Journal of Sytematic Bacteriology, Vol. 44, p. 285-292; Andou et al., US 5,252,469; Williams et al., Journal of
5 General Microbiology, Vol. 129, p. 1743-1813).

It is to be understood that also transferase variants are contemplated as the parent enzyme.

- 10 The activity of transglutaminases can be determined as described in "Methods of Enzymatic Analysis", third edition, 1984, Verlag Chemie, Weinheim, vol. 1-10.

Parent Phytases

15

Parent phytases are included in the group of enzymes classified under the Enzyme Classification number E.C. 3.1.3 (Phosphoric Monoester Hydrolases) in accordance with the Recommendations (1992) of the International Union of Biochemistry and Molecular
20 Biology (IUBMB)).

Phytases are enzymes produced by microorganisms which catalyse the conversion of phytate to inositol and inorganic phosphorus

- 25 Phytase producing microorganisms comprise bacteria such as *Bacillus subtilis*, *Bacillus natto* and *Pseudomonas*; yeasts such as *Saccharomyces cerevisiae*; and fungi such as *Aspergillus niger*, *Aspergillus ficuum*, *Aspergillus awamori*, *Aspergillus oryzae*, *Aspergillus terreus* or *Aspergillus nidulans*, and various other *Asper-*
30 *gillus* species).

Examples of parent phytases include phytases selected from those classified under the Enzyme Classification (E.C.) numbers: 3-phytase (3.1.3.8) and 6-phytase (3.1.3.26).

The activity of phytases can be determined as described in "Methods of Enzymatic Analysis", third edition, 1984, Verlag Chemie, Weinheim, vol. 1-10, or may be measured according to the method
5 described in EP-A1-0 420 358, Example 2 A.

Lyases

- 10 Suitable lyases include Polysaccharide lyases: Pectate lyases (4.2.2.2) and pectin lyases (4.2.2.10), such as those from *Bacillus licheniformis* disclosed in WO 99/27083.

Isomerases

15

Protein Disulfide Isomerase.

- Without being limited thereto suitable protein disulfide isomerases include PDIs described in WO 95/01425 (Novo Nordisk A/S) and suitable glucose isomerases include those described in Bio-
20 technology Letter, Vol. 20, No 6, June 1998, pp. 553-56.

Contemplated isomerases include xylose/glucose Isomerase (5.3.1.5) including Sweetzyme®.

Environmental allergens

25

The environmental allergens that are of interest for epitope mapping include allergens from pollen, dust mites, mammals, venoms, fungi, food items, and other plants.

- 30 Pollen, allergens include but are not limited to those of the order Fagales, Oleales, Pinales, Poales, Asterales, and Urticales; including those from *Betula*, *Alnus*, *Corylus*, *Carpinus*, *Olea*, *Phleum pratense* and *Artemisia vulgaris*, such as *Aln gl*,

Cor a1, Car b1, Cry j1, Amb a1 and a2, Art v1, Par j1, Ole e1, Ave v1, and Bet v1 (WO 99/47680).

Mite allergens include but are not limited to those from *Derm. farinae* and *Derm. pteronys.*, such as Der f1 and f2, and Der p1 and p2.

From mammals, relevant environmental allergens include but are not limited to those from cat, dog, and horse as well as from dandruff from the hair of those animals, such as Fel d1; Can f1; Equ c1; Equ c2; Equ c3.

Venue allergens include but are not limited to PLA2 from bee venom as well as Apis m1 and m2, Ves g1, g2 and g5, Ves v5 and te Pol and Sol allergens.

Fungal allergens include those from *Alternaria alt.* and *Cladospo. herb.* such as Alt a1 and Cla h1.

Food allergens include but are not limited to those from milk (lactoglobulin), egg (ovalbumin), peanuts, hazelnuts, wheat (alfa-amylase inhibitor),
Other plant allergens include latex (*hevea brasiliensis*).

In addition, a number of proteins of interest for expression in transgenic plants could be useful objects for epitope engineering. If for instance a heterologous enzyme is introduced into a transgenic plant e.g. to increase the nutritional value of food or feed derived from that plant, that enzyme may lead to allergenicity problems in humans or animals ingesting the plant-derived material. Epitope mapping and engineering of such heterologous enzymes or other proteins of transgenic plants may lead to reduction or elimination of this problem. Hence, the

methods of this patent are also useful for potentially modifying proteins for heterologous expression in plants and plant cells.

5

Materials and methods

Materials

10

ELISA reagents:

Horse Radish Peroxidase labelled pig anti-rabbit-Ig (Dako, DK, P217, dilution 1:1000).

Rat anti-mouse IgE (Serotec MCA419; dilution 1:100).

15 Mouse anti-rat IgE (Serotec MCA193; dilution 1:200).

Biotin-labelled mouse anti-rat IgG1 monoclonal antibody (Zymed 03-9140; dilution 1:1000)

Biotin-labelled rat anti-mouse IgG1 monoclonal antibody (Serotec MCA336B; dilution 1:2000)

20 Streptavidin-horse radish peroxidase (Kirkegård & Perry 14-30-00; dilution 1:1000).

Buffers and Solutions:

- PBS (pH 7.2 (1 liter))

25 NaCl 8.00 g

KCl 0.20 g

K₂HPO₄ 1.04 g

KH₂PO₄ 0.32 g

- Washing buffer PBS, 0.05% (v/v) Tween 20

30 - Blocking buffer PBS, 2% (wt/v) Skim Milk powder

- Dilution buffer PBS, 0.05% (v/v) Tween 20, 0.5% (wt/v) Skim Milk powder

- Citrate buffer 0.1M, pH 5.0-5.2

- Stop-solution (DMG-buffer)

- Sodium Borate, borax (Sigma)
 - 3,3-Dimethyl glutaric acid (Sigma)
 - Tween 20: Poly oxyethylene sorbitan mono laurate (Merck cat no. 822184)
 - 5 - PMSF (phenyl methyl sulfonyl flouride) from Sigma
 - Succinyl-Alanine-Alanine-Proline-Phenylalanine-paranitro-anilide (Suc-AAPF-pNP) Sigma no. S-7388, Mw 624.6 g/mol.
 - mPEG (Fluka)
- 10 Colouring substrate:
OPD: o-phenylene-diamine, (Kementec cat no. 4260)

Methods

15 Automatic epitope mapping

Implementation

The implementation consists of 3 pieces of code:

- 20 1. The core program (see above), written in C (see Appendix A).
2. A "wrapping" cgi-script run by the web server, written in Python (see Appendix B).
3. A HTML page defining the input/submission form (see Appen-
- 25 dix C).

The wrapper receives the input and calls the core program and several other utilities. Apart from the standard Unix utility programs (mv, rm , awk, etc..) the following must be installed:

30

- A web server capable of running cgi-scripts, eg. Apache
- Python 1.5 or later
- Gnuplot 3.7 or later

- DSSP, version July 1995

The core program

5

Inputs

1. A Brookhaven PDB file with the structure of the protein
2. The output of DSSP called with the above PDB file.
- 10 3. Maximum distance between adjacent residues
4. Minimum solvent accessible surface area for each residue
5. Maximum epitope size (max distance between any two residues in epitope)
6. Maximum number of non-redundant epitopes to include (0 =
15 all)
7. The shortest acceptable epitope (as a fraction of the length of the epitope consensus sequence).
8. Epitope consensus sequence describing which residues are possible at the different positions. An example is shown
20 below:

KR (Lys og Arg allowed)

AILV- (Ala, Ile, Leu, Val or missing residue allowed)

* (All residues allowed, but there must be a residue)

25 ? (All or missing residue allowed)

DE (Asp or Glu allowed)

(*, ? or - in first or last position is allowed but obsolete. (- in first position is ignored.))

30

Examples of matching epitopes:

KAAKD, KLASD, KLYSD, KLY-D, R-M-D.

The epitope searching algorithm

The "core" of the program is the algorithm that scans the protein surface for the epitope patterns. The principle is that several "trees" are built, where each of their branches describes one epitope:

1. All residues in the protein are checked according to:
10 a) Does the residue type match the first residue of the epitope consensus sequence. b) Is the surface accessibility greater than or equal to the given threshold. If both requirements are fulfilled, the protein residue is considered as one root in the epitope tree. Remark that there are usually many roots.
15
2. For each of the residues defined as roots, all residues within the the given threshold distance between adjacent residues (e.g. 7 Angstroms) are checked for the same as above:
20 a) Does the residue type match the second residue of the epitope consensus sequence. b) Is the surface accessibility greater than or equal to the given threshold. If yes, the protein residue is considered as a "child" of the root. The spatial position of a residue is defined as the coordinates of its C-alpha atom.
- 25 3. The procedure from step 2 is repeated for the next residue in the epitope consensus sequence, where each of the "childs" found in step 2 are now "roots" of new childs. If a gap is defined in the epitope consensus sequence, a "missing" residue is allowed, and the coordinates of the
30 root (also called "parent") is used.
4. This procedure is repeated for all residues in the epitope consensus sequence.

5. In this way a number of trees (corresponding to the number of roots found in step 1) are found. Notice that the same protein residue can be present many places in the trees.
6. If no epitopes that matches the length of the epitope consensus sequence are found, the longest shorter epitopes
5 that matches the first n residues of the epitope consensus sequence are used, where n is an integer smaller than the length of the epitope consensus sequence. If n is smaller than the length of the epitope consensus sequence multiplied by the fraction value defining the shortest acceptable epitope length, no epitopes are written to the output,
10 and steps 7, 8 and 9 are skipped.
7. The epitopes are extracted from the trees by traversing down from each of the "childs" in the last level. The algorithm also finds epitopes which have the same protein residue present more than once. This is, of course, an artifact and such epitopes are discarded. Every epitope is then checked for its size, that is, the maximum distance between any two residues which are members of the epitope. If this
15 exceeds the threshold, the epitope is discarded.
8. Redundant epitopes are removed. Epitopes containing one or more gaps are redundant if they are subsets of other epitopes without or with fewer gaps. For example: A82-gap-F45-G44-K43 is a subset of A82-L46-F45-G44-K43, and is therefore discarded.
20
9. For every epitope, the total solvent accessible surface area is calculated (by adding the contributions from each residue as found by the DSSP program). The epitopes are sorted according to this area in descending order. If a
25 maximum number of n non-redundant epitopes has been specified, the n epitopes with largest solvent accessible surface area are selected.
10. The output consists of a list of the found epitopes, along with information of the epitope consensus sequence used and
30

other internal parameters. A separate file containing the number of epitopes that each of the protein residues is a member of is also written.

5

The wrapper

Inputs

10

1. One PDB file, describing one structure, or one ZIP file, containing a number of PDB files, each describing one structure. The ZIP file must not contain subfolders.
2. An epitope consensus sequence or which part of the current
15 epitope library to use (full library or IgE part or IgG part).
3. Maximum distance between adjacent residues
4. Minimum solvent accessible surface area for each residue
5. Maximum epitope size (max distance between any two residues
20 in epitope)
6. Maximum number of non-redundant epitopes to include (0 = all)
7. Whether to use sequential numbering (1,2,3,4,..... etc) or PDB-file numbering.

25

Description

The core program accepts only one structure and one epitope consensus sequence. It is usually desirable to use a library of
30 epitope consensus sequences and sometimes several protein structures. The wrapper reads the user input and calls the utility programs and the core program the necessary number of times. The

output is collected and presented on the web page returned to the user.

Depending on the type of input, the wrapper works in different
5 modes:

- Epitope consensus can be given directly or taken from a library
 - Input type can be a single PDB file or a collection of PDB file given as a ZIP-file.
- 10 Any of the four possible combinations are allowed.

The epitope library consists of a number of text files, each containing one epitope consensus sequence as specified above.

15 The layout of the wrapper is like this:

1. Check if the program is already in use from somewhere else (this is done by checking for a lock file when the wrapper starts. If it does not exist, it is created and removed again when the program is finished).
- 20 2. If the epitope consensus sequences are to be read from the library, make an internal list of the desired library entries.
3. If the input type is a ZIP file, unzip the file and create one new directory for each of the contained PDB files. Move
25 each PDB file to its corresponding directory.
4. Do a loop over the structures and/or epitope consensus sequences. For each structure/epitope consensus sequence pair, DSSP and the core program is called with the required parameters. If the input type is a ZIP file, the outputs
30 are put in the appropriate directories.
5. If the epitope library is used, a sum file containing the total number of epitopes each residue is a member of. (Such a file is generated by the core program for each epitope consensus sequence - here a sum of these files is calcu-

lated). If input type is a ZIP file, a sum file is generated for each structure and put in the appropriate directory.

5 6. If the epitope library is used, a file containing the total number of epitopes found from each entry in the epitope library. If the input type is a PDB file, the file contains only one line (with a number of data corresponding to the library size). If the input type is a ZIP file, there is one line for each structure.

10 7. Depending on the combination of input type (ZIP or single PDB) and epitope consensus sequence source (typed-in or epitope library), different information is returned to the user:

15 Single PDB + typed in epitope: Graph of numbers of epitopes that each residue is a member of. List of found epitopes.

ZIP file + typed in epitope: Graphs (one for each structure) of numbers of epitopes that each residue is a member of. Lists (one for each structure) of found epitopes.

20 Single PDB + epitope library: Graph of numbers of epitopes that each residue is a member of (total for the complete library).

25 ZIP file + epitope library: Graphs (one for each structure) of numbers of epitopes that each residue is a member of (total for the complete library).

Data flow sheets for the four different are shown in the figure

30 8. For all modes except Single PDB + typed in epitope, a ZIP file containing all output files is created and returned to the user.

Immunisation of Brown Norway rats:

Twenty intratracheal (IT) immunisations were performed weekly with 0,100 ml 0.9% (wt/vol) NaCl (control group), or 0,100 ml of a protein dilution (~0,1-1 mg/ml). Each group contained 10 rats. Blood samples (2 ml) were collected from the eye one week after every second immunisation. Serum was obtained by blood clotting and centrifugation and analysed as indicated below.

10 Immunisation of Balb/C mice:

Twenty subcutaneous (SC) immunisations were performed weekly with 0.05 ml 0.9% (wt/vol) NaCl (control group), or 0,050 ml of a protein dilution (~0,01-0,1 mg/ml). Each group contained 10 female Balb/C mice (about 20 grams) purchased from Bomholdtgaard, Ry, Denmark. Blood samples (0,100 ml) were collected from the eye one week after every second immunisation. Serum was obtained by blood clotting and centrifugation and analysed as indicated below.

20 ELISA Procedure for detecting serum levels of IgE and IgG:

Specific IgG1 and IgE levels were determined using the ELISA specific for mouse or rat IgG1 or IgE. Differences between data sets were analysed by using appropriate statistical methods.

25

Activation of CovaLink plates:

A fresh stock solution of cyanuric chloride in acetone (10 mg/ml) is diluted into PBS, while stirring, to a final concentration of 1 mg/ml and immediately aliquoted into CovaLink NH₂ plates (100 microliter per well) and incubated for 5 minutes at room temperature. After three washes with PBS, the plates are

dried at 50°C for 30 minutes, sealed with sealing tape, and stored in plastic bags at room temperature for up to 3 weeks.

Mouse anti-Rat IgE was diluted 200x in PBS (5 microgram/ml). 100
5 microliter was added to each well. The plates were coated overnight at 4 °C.

Unspecific adsorption was blocked by incubating each well for 1 hour at room temperature with 200 microliter blocking buffer.
10 The plates were washed 3x with 300 microliter washing buffer.

Unknown rat sera and a known rat IgE solution were diluted in dilution buffer: Typically 10x, 20x and 40x for the unknown sera, and ½ dilutions for the standard IgE starting from 1
15 µg/ml. 100 microliter was added to each well. Incubation was for 1 hour at room temperature.

Unbound material was removed by washing 3x with washing buffer. The anti-rat IgE (biotin) was diluted 2000x in dilution buffer.
20 100 microliter was added to each well. Incubation was for 1 hour at room temperature. Unbound material was removed by washing 3x with washing buffer.

Streptavidin was diluted 1000x in dilution buffer. 100 microliter was added to each well. Incubation was for 1 hour at room temperature. Unbound material was removed by washing 3x with 300 microliter washing buffer. OPD (0.6 mg/ml) and H₂O₂ (0.4 microliter /ml) were dissolved in citrate buffer. 100 microliter was added to each well. Incubation was for 30 minutes at room temperature.
30 perature. The reaction was stopped by addition of 100 microliter H₂SO₄. The plates were read at 492 nm with 620 nm as reference.

Similar determination of IgG can be performed using anti Rat-IgG and standard rat IgG reagents.

- 5 Similar determinations of IgG and IgE in mouse serum can be performed using the corresponding species-specific reagents.

Direct IgE assay:

- 10 To determine the IgE binding capacity of protein variants one can use an assay, essentially as described above, but using sequential addition of the following reagents:
- 1) Mouse anti-rat IgE antibodies coated in wells;
 - 15 2) Known amounts of rat antiserum containing igE against the parent protein;
 - 3) Dilution series of the protein variant in question (or parent protein as positive control);
 - 4) Rabbit anti-parent antibodies
 - 20 5) HRPO-labelled anti-rabbit Ig antibodies for detection using OPD as described.

The relative IgE binding capacity (end-point and/or affinity) of the protein variants relative to that of the parent protein are
25 determined from the dilution-response curves. The IgE-positive serum can be of other animals (including humans that inadvertently have been sensitized to the parent protein) provided that the species-specific anti-IgE capture antibodies are changed accordingly.

30

Competitive ELISA (C-ELISA):

C-ELISA was performed according to established procedures. In short, a 96 well ELISA plate was coated with the parent protein.

After proper blocking and washing, the coated antigen was incubated with rabbit anti-enzyme polyclonal antiserum in the presence of various amounts of modified protein (the competitor). The residual amount of rabbit antiserum was detected by horseradish peroxidase-labelled pig anti-rabbit immunoglobulin.

Protein sequences and alignments:

For purposes of the present invention, the degree of homology may be suitably determined by means of computer programs known in the art, such as GAP provided in the GCG program package (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711) (Needleman, S.B. and Wunsch, C.D., (1970), Journal of Molecular Biology, 48, 443-45).

Subtilisin proteases:

In the present invention, corresponding (or homologous) positions in subtilisin protease sequences are defined by alignment with Subtilisin Novo (BPN') from *B.amyloliquefaciens*, as shown in Table 1A for Alcalase, Protease B, Esperase, Protease C, Protease D, Protease E, Protease A, PD498, Properase, Relase, Savinase.

Table 1A: Alignment of different proteases to the sequence of BPN'

30 Alcalase:

69.5% identity in 275 residues overlap; Score: 953.0; Gap frequency: 0.4%

Alcalase,	1	AQTVPYGIPLIKADKVAQGFPGANVKVAVLDTG IQASHFDLNVVGGASFVAGEAYN-TD
BPN',	1	AQSVPYGVSIKAPALHSQGYTGSENVKVAVIDSGIDSSHPDLKVAGGASMVPSETPNFQD
35		** ***** *** ** * ***** * * * ***** * * * * *
Alcalase,	60	GNHGTHVAGTVAALDNTTGVILGVAPSVSLYAVKVLNSSGSGSYSGIVSGIERATTNGMD

118

```

BPN',          61 DNSHGTHVAGTVAALNNSIGVLGVAPSSALYAVKVLGDAGSGQYSWIINGIEWAIANNMD
                * * * * *
Alcalase,      120 VINMSLGGASGSTAMKQAVDNAYARGVVVVAAGNSGSSGNTNTIGYPAKYDSVIAVGAV
5 BPN',         121 VINMSLGGPSGSAALKAADVDAVASGVVVVAAGNEGSTGSSSTVGYPGKYPSVIAVGAV
                * * * * *
Alcalase,      180 DSNNSNRASFSSVGAELEVMAPGAGVYSTYPTNTYATLNGTSMASPHVAGAAALILSKHPN
BPN',          181 DSSNQRAFSSVSGPELDVMAPGVS IQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPN
10             ** * * * *
Alcalase,      240 LSASQVRNRLSSTATYLGSSFFYKGGLINVEAAAQ
BPN',          241 WTNTQVRSSLQNTTTKLGDSFFYKGGLINVQAAAQ
                * * * * *
15

```

Protease B:

59.6% identity in 275 residues overlap; Score: 820.0; Gap frequency: 2.2%

```

20 PROTEASE B,      1 AQTIPWGISRVQAPAAHNRLTGSQVKAVALDTGI-STHPDLNIRGGASFVPGE-PTQD
   BPN',           1 AQSVPYGVSGQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETPNFQD
                   ** * * *   * * *   * * *   * * *   * * *   * * *   * * *
25 PROTEASE B,      59 GNGHGTHTVAGTIAALNNSIGVLGVAPSAELYAVKVLGASGSGSVSSIAQGLEWAGNNGMH
   BPN',           61 DNSHGTHTVAGTVAALNNSIGVLGVAPSSALYAVKVLGDAGSQYSWIINGIENAIANNMD
                   * * * * *   * * * * *   * * * * *   * * *   * *   * * *
30 PROTEASE B,      119 VANLSLGSPSPSATLEQAVNSATSRGVLVVAASGNsga---GSISYPARYANAMAVGAT
   BPN',           121 VINMSLGGPSSGAALKAAVDKAVASGVVVVAAAGNEGSTGSSSTVGYPGKYPSVIAVGAV
                   * * * * *   * * *   * * *   * * *   * * *   * * *   * * *
35 PROTEASE B,      175 DONNNRASFQYAGALDIMPAGVNIQSTYPGSTYASDNGTSMATPHVAGAAALVKQKNPS
   BPN',           181 DSSNQRAFSSVSGPELDVMAPGVS IQSTLPGNKYGA YNGTSMASPHVAGAAALILSKHPN
                   * * * * *   * * * * *   * * * * *   * * *   * * *   * *
40 PROTEASE B,      235 WSNVQIRNHLKNTATSLGSTNLYGSGLVNAEAATR
   BPN',           241 WTNTQVRSSIQNTTTKLGDsfyygkGLINVQAAAQ
                   * * * * *   * * * * *   * * * * *   * * *   * * *

```

Esperase:

54.7% identity in 274 residues overlap; Score: 745.0; Gap frequency: 2.2%

```

45  Esperase,      1  QTVPWGISFINTQQAHRNGIFGNGARVAVLDTGI-ASHPDLRIAGGASFISSE-PSYHDN
    BPN',          2  QSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETPNFQDD
                        * * * * *      * * *      * * * * *      * * * * *      * * * *
    Esperase,      59  NGHGHVAGTIAALNNSIGVLGVAPADLYAVKVLDRNGSGSLASVAQGI EWAINNNMHI
50  BPN',          62  NSHGHVAGTVAALNNSIGVLGVAPSSALYAVKVLGDAGSGQYSWIINGI EWAIANNMDV
                        * * * * *      * * * * *      * * *      * * * * *      * * *
    Esperase,      119 INMSLGSTSGSSTLELAVNRANNAGILLVGAAGNTGRQG---VNYPARYSGVMVAVAVD
    BPN',          122 INMSLGSPSGSAALKAADV KAVAGSVVVAAAGNEGSTGSSSTVGYPGKYPSVI AVGAVD
55                        * * * * *      * * *      * * *      * * * * *      * * * * *      * * * * *
    Esperase,      175 QNGQRASFSTYGP EIEISAPGVNVNSTYTGNRYVSLSGTSMATPHVAGVAALVKSRYPsy
    BPN',          182 SSNQRASFSVSGPELDVMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPNW
                        * * * * *      * * *      * * *      * * *      * * * * *      * * *
60  Esperase,      235 TNNQIRQRINQTATYLGSPSLYGNGLVHAGRATQ
    BPN',          242 TNTQVRSSLQNTTTKLGDSPFYKGLINQVAAAQ
                        * * *      * * *      * * *      * * *      * * *

```

Protease C:

65 59.6% identity in 275 residues overlap; Score: 825.0; Gap frequency: 2.2%

ProteaseC, 1 AQSVPWGISRVOAPAAHNRLTGSGVRVAVLDTGI-STHPDLNIRGGASFVPGGE-PSTOD

119

BPN', 1 AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHDLKVAGGASMVPSETPNFQD

ProteaseC, 59 GNGHGHVAGTIAALNNSIGVLGVAPSABLYAVKVLGASGSGSYSSIAQGLEWAGNNGMH
 5 BPN', 61 DNSHGTHVAGTVAALNNSIGVLGVAPSSALYAVKVLGDAGSGQYSWIINGIEWAIANNMD

ProteaseC, 119 VASLSLGSPPSATLEQAVNSATSRGVLVVAASGNSGA----GSISYPARYANAMAVGAT
 BPN', 121 VINMSLGGPSGSAALKAADVAVASGVVVVAAAGNEGSTGSSSTVGYPGKYPVIAVGAV
 10 *****

ProteaseC, 175 DQNNNRASFQYAGLDIVAPGVNVQSTYPGSTYASLNGTSMATPHVAGAAALVKQKNPS
 BPN', 181 DSSNQRAFSSVGPEDLVMAPGVSIGSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPN

15
 ProteaseC, 235 WSNVQIRNHLKNTATSLGSTNLYGSGLVNAEAAAR
 BPN', 241 WTNTQVRSSLQNTTTKLGDSEFYGKGLINVQAAAQ

20

Protease D:
 59.3% identity in 275 residues overlap; Score: 815.0; Gap frequency: 2.2%

25 ProteaseD, 1 AQSVPWGISRVQAPAAHNRGLTSGGVKVAVLDTGI-STHPDLNIRGGASFVPGE-PSTQD
 BPN', 1 AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHDLKVAGGASMVPSETPNFQD

ProteaseD, 59 GNGHGHVAGTIAALNNSIGVLGVAPSABLYAVKVLGASGSGAISSIAQGLEWAGNNGMH
 30 BPN', 61 DNSHGTHVAGTVAALNNSIGVLGVAPSSALYAVKVLGDAGSGQYSWIINGIEWAIANNMD

ProteaseD, 119 VANLSLGSPPSATLEQAVNSATSRGVLVVAASGNSGA----GSISYPARYANAMAVGAT
 BPN', 121 VINMSLGGPSGSAALKAADVAVASGVVVVAAAGNEGSTGSSSTVGYPGKYPVIAVGAV
 35 *****

ProteaseD, 175 DQNNNRASFQYAGLDIVAPGVNVQSTYPGSTYASLNGTSMATPHVAGAAALVKQKNPS
 BPN', 181 DSSNQRAFSSVGPEDLVMAPGVSIGSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPN

40
 ProteaseD, 235 WSNVQIRNHLKNTATSLGSTNLYGSGLVNAEAAAR
 BPN', 241 WTNTQVRSSLQNTTTKLGDSEFYGKGLINVQAAAQ

45

Protease E:
 58.2% identity in 275 residues overlap; Score: 800.0; Gap frequency: 2.2%

ProteaseE, 1 AQSVPWGISRVQAPAAHNRGLTSGGVKVAVLDTGI-STHPDLNIRGGASFVPGE-PSTQD
 50 BPN', 1 AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHDLKVAGGASMVPSETPNFQD

ProteaseE, 59 GNGHGHVAGTIAALNNSIGVLGVAPSABLYAVKVLGASGSGAISSIAQGLEWAGNNGMH
 BPN', 61 DNSHGTHVAGTVAALNNSIGVLGVAPSSALYAVKVLGDAGSGQYSWIINGIEWAIANNMD
 55 *****

ProteaseE, 119 VANLSLGSPPSATLEQAVNSATSRGVLVVAASGNSGA----DSISYPARYANAMAVGAT
 BPN', 121 VINMSLGGPSGSAALKAADVAVASGVVVVAAAGNEGSTGSSSTVGYPGKYPVIAVGAV

60
 ProteaseE, 175 DQNNNRASFQYAGLDIVAPGVNVQSTYPGSTYASLNGTSMATPHVAGAAALVKQKNPS
 BPN', 181 DSSNQRAFSSVGPEDLVMAPGVSIGSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPN

65 ProteaseE, 235 WSNVRI RDHLKKTATSLGSTNLYGSGLVNAEAAAR
 BPN', 241 WTNTQVRSSLQNTTTKLGDSEFYGKGLINVQAAAQ

Protease A:

5 58.9% identity in 275 residues overlap; Score: 812.0; Gap frequency: 2.2%

Protease A, 1 AQSVPWGISRVQAPAAHNRGLTSGGVKVAVLDTGI-STHPDLNIRGGASFVPGE-PSTQD
BPN', 1 AQSVPYGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPLKLVAGGASMVPSETPNFQD
***** * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

10 Protease A, 59 GNGHGHVAGTIAALNNSIGVLGVAPSAELYAVKVLGASGSGSVSSIAQGLEWAGNNGMH
BPN', 61 DNSHGHVAGTVAALNNSIGVLGVAPSSALYAVKVLGDAGSGQYSWIINGIEWAIANNMD
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

15 Protease A, 119 VANLSLGSPSAGGTLEQAVNSATSRGVLVVAASGNSGA---GSISAPASYANAMAVGAT
BPN', 121 VINMSLGGPSGSAALKAAVDKAVASGVVVAAAGNEGSTGSSSTVGYPGKYPSVIAVGAV
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

Protease A, 175 DQNNNRASFQYGPGLDIVAPGVNVQSTYPGSTYASLNGTSMATPHVAGAAALVKQKNPS
20 BPN', 181 DSSNQRAFSSVGPELDVMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPN
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

Protease A, 235 WSNVQIRNHLKNTATSLGSTNLYGSLVNAEAATR
BPN', 241 WINTQVRSSLQNTTTKLGDSPFYKGGLINVQAAQ
25 * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

PD498:

47.7% identity in 266 residues overlap; Score: 487.0; Gap frequency: 4.9%

PD498, 13 YGPQNTSTPAAWDVTRGSSTQTVAVLDSGVDYNHPDLARKVIKGYDFIDRDN-NPMDLNG
 5 BPN', 6 YGVSQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDL--KVAGGASMVPSETPNFQDDNS
 ** ** * ** * ** * ** * ** * ** * ** * ** *

PD498, 72 HGTHVAGTVAADTNNGIGVAGMAPDTKILAVRVLDANGSGSLDSIASGIRYAADQGAQVL
 10 BPN', 64 HGTHVAGTVAA-INNSIGVLGVAPSSALYAVKVLGDAGSGQYSWIINGIEWAIANNMDVI
 ***** ** * ** * ** * ** * ** * ** * ** *

PD498, 132 NLSLGCECNSTTLKSAVDYAWNKGAVVVAAGND---NVSRTFQPASYPNAIIVGAIDS
 BPN', 123 NMSLGGPSSGSAALKAADVKAASGVVVVAAGNEGSGTSSSTVGYPGKYPVIAVGAIDS
 * ** * ** * ** * ** * ** * ** * ** * ** *

15 PD498, 188 NDRKASFSNYGTWVDVTAPGVNIASITVPNNGYSYMSTSMASPHVAGLAALASQCKN--
 BPN', 183 SNQASFSVSGPELDMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPNWT
 ***** * ** * ** * ** * ** * ** * ** * ** *

20 PD498, 246 NVQIRQAIEQTADKISGTGTFKYGK
 BPN', 243 NTQVRSSLQNTTTKL---GDSFYYGK
 * ** * ** * ** * ** * ** *

25

Properase:

58.9% identity in 275 residues overlap; Score: 813.0; Gap frequency: 2.2%

Properase, 1 AQSVPWGISRVQAPAAHNRGLTSGGVKVAVLDTGI-STHPDLNIRGGASFVPGE-PSTQD
 30 BPN', 1 AQSVPYGVSVQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETPNFQD
 ***** * ** * ** * ** * ** * ** * ** * ** *

Properase, 59 GNGHGTHVAGTIAALNNSIGVLGVAPNAELYAVKVLGASGGGSSNSIAQGLEWAGNNGMH
 35 BPN', 61 DNSHGTHVAGTVAALNNSIGVLGVAPSSALYAVKVLGDAGSGQYSWIINGIEWAIANNMD
 * ***** ** * ** * ** * ** * ** * ** * ** *

Properase, 119 VANLSLGSPSPSATLEQAVNSATSRGVLVVAASGNSGA---GSISYPARYANAMAVGAT
 BPN', 121 VINMSLGGPSSGSAALKAADVKAASGVVVVAAGNEGSGTSSSTVGYPGKYPVIAVGA
 * ** * ** * ** * ** * ** * ** * ** * ** *

40 Properase, 175 DQNNRASFSQYGAGLDIVAPGVNVQSTYPGSTYASLNGTSMATPHVAGAAALVKQKNPS
 BPN', 181 DSSNQASFSVSGPELDMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPN
 * * ***** * ** * ** * ** * ** * ** * ** *

45 Properase, 235 WSNVQIRNHLKNTATSLGSTNLGSGLVNAEAAATR
 BPN', 241 WTNQVRSSLQNTTTKLGDSTFYGKGLINVAQAAQ
 * * ** * ** * ** * ** * ** * ** *

50 Relase:

60.7% identity in 275 residues overlap; Score: 858.0; Gap frequency: 1.8%

Relase, 1 AQSVPWGISRVQAPAAHNRGLTSGGVKVAVLDTGIDSTHPDLNIRGGASFVPGE-PSTQD
 55 BPN', 1 AQSVPYGVSVQIKAPALHSQGYTGSNVKVAVIDSGIDSSHPDLKVAGGASMVPSETPNFQD
 ***** * ** * ** * ** * ** * ** * ** * ** *

Relase, 60 GNGHGTHVAGTIAALDNSIGVLGVAPSAELYAVKVLGASGSGSVSSIAQGLEWAGNNGMD
 BPN', 61 DNSHGTHVAGTVAALANSIGVLGVAPSSALYAVKVLGDAGSGQYSWIINGIEWAIANNMD
 * ***** ** * ** * ** * ** * ** * ** * ** *

60 Relase, 120 VANLSLGSPSPSATLEQAVNSATSRGVLVVAASGNSGA---GSISYPARYANAMAVGAT
 BPN', 121 VINMSLGGPSSGSAALKAADVKAASGVVVVAAGNEGSGTSSSTVGYPGKYPVIAVGA
 * * ** * ** * ** * ** * ** * ** * ** *

65 Relase, 176 DQNNRASFSQYGAEIDIVAPGVNVQSTYPGSTYASLNGTSMATPHVAGAAALVLQKNPS
 BPN', 181 DSSNQASFSVSGPELDMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPN
 * * ***** * ** * ** * ** * ** * ** * ** *

Release,	236	WSNVQIRNHLKNTATSLGSTNLYGSGLVNAEAATR
BPN',	241	WTNTQVRSSLQNTTTKLGDSEFYFGKGLINVQAAAQ
		* * * * *
5		
<u>Savinase:</u>		
59.6% identity in 275 residues overlap; Score: 821.0; Gap frequency: 2.2%		
10		
Savinase,	1	AQSVPWGISRVQAPAAHNRGLTSGGVKVAVLDTGI-STHPDLNIRGGASFVPGE-PSTQD
BPN',	1	AQSVPYGVSQIKAPALHSQGYTGSENVKVAVIDSGIDSSHPDLKVAGGASMVPSETPNFQD

15		
Savinase,	59	GNHGHTHVAGTIAALNNSIGVLGVAPSAELYAVKVLGASGSGSVSSIAQGLEWAGNNGMH
BPN',	61	DNSHGHTHVAGTVAALNNSIGVLGVAPSSALYAVKVLGDAGSCQYSWIINGIEWAIANNMD
		* * * * *
20		
Savinase,	119	VANLSLGSPPSPSATLEQAVNSATSRGVLVVAASGNSGA---GSISYPARYANAMAVGAT
BPN',	121	VINMSLGGPSCGAALKAADVKAASGVVVVAAAGNEGSTGSSSTVGYPGKYPSVIAVGAV
		* * * * *
25		
Savinase,	175	DONNNRASFSQYGAGLDIVAPGVNVQSTYPGTYASLNGTSMATPHVAGAAALVKQKNPS
BPN',	181	DSSNQRAFSSVGPGLDVMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPN
		* * * * *
30		
Savinase,	235	WSNVQIRNHLKNTATSLGSTNLYGSGLVNAEAATR
BPN',	241	WTNTQVRSSLQNTTTKLGDSEFYFGKGLINVQAAAQ
		* * * * *

To find the homologous positions in subtilisin protease sequences not shown in the alignment of Table 1A, the sequence of interest is aligned to the sequence of BPN' as shown in Table 1B for YaB protease and Subtilisin sendai. The new sequence is aligned to the BPN' sequence by using the GAP alignment to the most homologous sequence found by the GAP program. GAP is provided in the GCG program package (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711) (Needleman, S.B. and Wunsch, C.D., (1970), Journal of Molecular Biology, 48, 443-45).

The sequence of the YaB protease is disclosed by Kaneko, R.; Koyama, N.; Tsai, Y.-C.; Juang, R.-Y.; Yoda, K.; Yamasaki, M.; Molecular cloning of the structural gene for alkaline elastase YaB, a new subtilisin produced by an alkalophilic Bacillus strain. J.

The sequence of the Subtilisin sendai is disclosed by Yamagata, Y.; Isshiki, K.; Ichishima, E.; Subtilisin Sendai from alkalophilic *Bacillus* sp.: molecular and enzymatic properties of the enzyme and molecular cloning and characterization of the gene, *aprS*. *Enzyme Microb. Technol.* 17:653 (1995), it has SPTREMBL accession number Q45522, and is shown in SEQ ID NO 34.

10

identity to savinase: 82,09%

15 Swissprot: P20724

Table 1B:

20 Alignment of YAB protease to BPN': 55,3% identity
CLUSTAL W (1.7) multiple sequence alignment

```

YAB      -QTVPGWGINRVQAPIAQSRGFTGTGVRVAVLDTGISN-HADLRIRGGASFVPGE-PNISD
25 BPN~   AQSVPYPYGVSGQIKAPALHSGQYTGSNVKVAVIDSGIDSSHPDLKVAGGASMPVSETPNFQD
          *::*:::*:::*:::*:::*:::*:::*:::*:::*:::*:::*:::*:::*:::*:::*
          *::*:::*:::*:::*:::*:::*:::*:::*:::*:::*:::*:::*:::*:::*

YAB      GNGHGTQVAGTTAAALNNSIGVLGVAPNVDLYGVKVLGASGSGSISGIAQGLQWAANNMGH
30 BPN~   DNSHGHTVAGTVAALNNSIGVLGVAPSSALYAVKVLGDAGSGQYSWIINGIIEWAIANNMD
          .*:::*:::*:::*:::*:::*:::*:::*:::*:::*:::*:::*:::*:::*
          .*:::*:::*:::*:::*:::*:::*:::*:::*:::*:::*:::*:::*:::*

YAB      IANMSLGSSAGSATMEQAVNQATASGVLVVAASGNSG----AGNVGFPARYANAMAVGAT
BPN~     VINMSLGGPSSGAALKAADVKAVASGVVVVAAGKVDTSGSSTVGYGPKYPSVIAVGAV
          :::*:::*:::*:::*:::*:::*:::*:::*:::*:::*:::*:::*:::*
          :::*:::*:::*:::*:::*:::*:::*:::*:::*:::*:::*:::*:::*

35 YAB      DONNNRATFSQYGAGLDIVAPGVGVQSTVPNGNYASFNGTSMATPHVAGVAALVKQKNPS
BPN~     DSSNQRASFPSSVGPPELDMVAPGVSIQSTLPGNKYGA YNGTSMASPHVAGAAALILSKHPN
          *..*:::*:::*. *. *::*:::*:::*:::*:::* *.:::*:::*:::*:::*:::*:::*
          *..*:::*:::*. *. *::*:::*:::*:::*:::* *.:::*:::*:::*:::*:::*:::*

40 YAB      WSNVQIRNHLKNTATNLGNMTTQFGSLVNAEAATR
BPN~     WINTQVRSSLQNTTTKLGDSFYFGKGLINVAQAAQ
          **..*:::*. *::*:::*:::*:::*:::* *::*:::*:::*
          **..*:::*. *::*:::*:::*:::*:::* *::*:::*:::*

```

45

Alignment of Subtilisin sendai to BPN': 55,6% identity.
CLUSTAL W (1.7) multiple sequence alignment

```

50 sendai      NQVTPWGITRVOAPTAWTEGTYGTGVRVAVLDTGIS-THPDLNIRGGVSFVPGE-PSYQD
BPN^          AQSVPYPGVSGIKAPALHSQGYFGSNNKVAVIDSGIDSSHPDLKVAGGASMPVSETPNFQD
              * .*:.::::**: ::****:.*;***:*:*.. :*****: ***.**.* *.**

sendai        GNGHGTHVAGTIAALNNSIGVVGVAPNAELIYAVVKVLGANGSGSVSSIAQGLOWTAQNIIH
55 BPN^        DNSHGTHVAGTVAALNNSIGVLGVAPSSALYAVVKVLGDAGSGQYSWIINGISWATANNMD
              .*****:***** ***: ***** ***. *.*.....**

```

```

sendai      VANLSLGPSPVGSQTLLEAVNQATNAGVLVVAATGNNG----SGTVSYPPARYANALAVGAT
BPN~        VINMSLGGPSPGSAALKAAVDKAVASGVVVVAAAGNEGSGSSSTVGYPGKYPVIAVGAV
            * *:***: * * *: *: **: *: *: **:***:***: * * *:***:***:***:
5 sendai     DQNNNRASFQSYGTGLNIVAPGVGIQSTYPGNRYASLSGTSMATPHVAGVAALVKQKNPS
BPN~        DSSNQRASFSVSGPELDVMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKRPN
            * *:***:***: * *:***:***:***:***:***: * *:***:***:***:***:
sendai      WSNTOIRQHLTSTATSLGNSNQFGSGLVNAEATR
10 BPN~      WTNTOVRSSLQNTTTKLGDSPFYQKGLINQAAAQ
            * *:***:***: * *:***:***:***:***:***:

```

These alignments reveal that that homology between various sub-
15 tilisin proteases ranges between 100% and 40%.

Unless specified, subtilisin sequences and positions mentioned in the present invention, are given in the BPN' numeration, and can be converted by alignment as described above (Tables 1A and 20 1B).

Sequence identities between different pairs of proteases are given below:

25 Sequence identity to BPN':

	Savinase	60.4%
	Alcalase	69.5%
	BLAPR	60.4%
	ProteaseC	60.4%
30	ProteaseD	60.0%
	ProteaseE	58.2%
	Protease A	60.0%
	Properase	59.6%
	Relase	61.5%
35	PD498	44.8%
	sendai	55.6%
	YAB	55.3%

Sequence identity to Savinase:

40 Alcalase 60.9%

125

	BLAPR	98.1%
	ProteaseC	98.5%
	ProteaseD	98.9%
	ProteaseE	96.7%
5	Protease A	97.8%
	Properase	98.9%
	Relase	98.1%
	PD498	44.3%
	sendai	81.4%
10	YAB	81.8%

Structures

The protein structure of PD498 is disclosed in WO98/35026 (Novo
15 Nordisk). The structure of Savinase can be found in BETZEL et al,
J.MOL.BIOL., Vol. 223, p. 427, 1992 (1svn.pdb).

Homology modelling

20 Three dimensional structural models of the subtilisins prop-
erases, relase, ProteaseC, ProteaseD, ProteaseE, and PROTEASE B
were constructed based on three dimensional structure of Savi-
nase (Protein Data Bank entry 1SVN; Betzel, C., Klupsch, S.,
Papendorf, G., Hastrup, S., Branner, S., Wilson, K. S.: Crystal
25 structure of the alkaline proteinase Savinase from *Bacillus len-*
tus at 1.4 Å resolution. *J Mol Biol* 223 pp. 427 (1992)) using
the Modeller 5.0 (Šali, A.; T.L. Blundell, "Definition of general
topological equivalence in protein structures: A procedure in-
volving comparison of properties and relationships through simu-
30 lated annealing and dynamic programming," *J. Mol. Biol.*, 212
403-428 (1990)) module of the Insight 2000 molecular modelling
package (Biosym inc.). Default parameters were used with the
alignments shown in Figure 1A as input, e.g. alignment between
the columns labelled Savinase and PROTEASE B served as input

alignment in construction of a PROTEASE B structural model. The Modeller module by default output ten structural models, of these the model with lowest 'modeller objective function' score was chosen as representing PROTEASE B structure.

5

Lipase:

The sequence of the *T. lanuginosus* lipase (trade name Lipolase) is provided in SEQ ID NO 1 and the structure is disclosed in WO 98/35026 and as "1tib", available in Structural Classification of Proteins (SCOP) on the Internet..

15 Amylase:

The amylase used in the examples is the alpha-amylase of *Bacillus halmapalus* (WO96/23873), which is called amylase SP722 (the wild-type). Its sequence is shown in SEQ ID NO 2 and the corresponding protein structure was built from the BA2 structure, as described in WO96/23874. The first four amino acids of the structural model are not defined, hence the sequence used for numeration of amino acid residues in the examples of this invention is four amino acids shorter than the one of the full length protein SP722.

25

Several variants of this amylase are available (WO96/23873). One particularly useful variant has deleted two amino acid residues at D-G at positions 183 and 184 of the SEQ ID NO 2 (corresponding to residues 179 and 180 of the modelled structure). This variant is called JE-1 or Natalase.

30

Another amylase that is particularly useful is the amylase AA560: This alkaline α -amylase may be derived from a strain of *Bacillus* sp. DSM 12649. The strain was deposited on 25th January 1999 by

the assignee under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure at Deutsche Sammlung von Microorganismen und Zellkulturen GmbH (DSMZ), Mascheroder Weg 1b, D-38124
5 Braunschweig DE.

Laccase:

10 The laccase used in this invention is that from *Coprinus cinereus* (WO98/38287), the sequence of which is shown as SEQ ID NO 3. The structure of the *Myceliophthora thermophila* laccase can be built by homology modeling to the *Coprinus cinereus* laccase as shown in WO98/38287.

15

Cellulase:

The cellulase sequence and structure used in the present invention is that of the core fragment of endoglucanase V from *Humicola insolens* (aka Cel45 or Carezyme). The core fragment structure is available as 3eng.pdb (G.J.DAVIES et al. ACTA CRYSTALLOGR., SECT.D, Vol. 52, p.7 1996; G.J.DAVIES et al. BIOCHEMISTRY, V. 34, p. 16210, 1995); SwissProt accession number P43316, and the sequences shown in SEQ ID 4. The corresponding full-length
25 sequence is disclosed in WO91/17243 and shown here in SEQ ID NO 5. The numeration of all description and claims of this invention pertain to the core fragment, however, it is contemplated that all claims are also valid for the corresponding positions in the full-length protein.

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T R G S S T Q T V A V L D S G V D Y N H P D L A R K V I K
 G L T G S G V K V A V L D T G - S T H P D L N - R G
 G L T G S G V K V A V L D T G - D S T H P D L N - R G
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 G L T G S G V K V A V L D T G - S T H P D L N - R G
 G L T G S G V R V A V L D T G - S T H P D L N - R G
 G I F G N G A R V A V L D T G - A S H P D L R - A G
 G L T G S G V K V A V L D T G - S T H P D L N - R G
 G F K G A N V K V A V L D T G - Q A S H P D L N V V G
 G Y T G S N V K V A V I D S G I D S S H P D L K V A G

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G Y D F - I D R D N N R M D L N G H G T H V A G T V A A D T

G A S F V P G E R S T Q D G N G H G T H V A G T - A A L

G A S F V P G E R S T Q D G N G H G T H V A G T - A A L

G A S F V P G E R S T Q D G N G H G T H V A G T - A A L

G A S F V P G E R S T Q D G N G H G T H V A G T - A A L

G A S F V P G E R S T Q D G N G H G T H V A G T - A A L

G A S F V P G E R S T Q D G N G H G T H V A G T - A A L

G A S F V P G E R S T Q D G N G H G T H V A G T - A A L

G A S F I S S E P S Y H D N N G H G T H V A G T - A A L

G A S F V P G E R S T Q D G N G H G T H V A G T - A A L

G A S F V A G E A Y N T D G N G H G T H V A G T V A A L

G A S M V P S E T P N F Q D D N S H G T H V A G T V A A L

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NNG - G V A G M A P D T K - L A V R V L D A N G S G S L D

NNS - G V L G V A P S A E L Y A V K V L G A S G S G S V S

DNS - G V L G V A P S A E L Y A V K V L G A S G S G S V S

NNS - G V L G V A P N A E L Y A V K V L G A S G G G S N S

NNS - G V L G V A P S A E L Y A V K V L G A S G S G S V S

NNS - G V L G V A P S A E L Y A V K V L G A S G G G A I S

132 DNS - G V L G V A P S A E L Y A V K V L G A S G S G A I S

NNS - G V L G V A P S A E L Y A V K V L G A S G S G S Y S

NNS - G V L G V A P S A D L Y A V K V L D R N G S G S L A

NNS - G V L G V A P S A E L Y A V K V L G A S G S G S V S

DNTT - G V L G V A P S V S L Y A V K V L N S S G S G S Y S

NNS - G V L G V A P S S A L Y A V K V L G D A G S G Q Y S

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S I A Q G L E W A G N N G M H V A N L S L G S P S P S A T L

S I A Q G L E W A G N N G M H V A N L S L G S P S A G G T L

S I A Q G L E W A G N N G M H V A N L S L G S P S P S A T L

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E Q A V N S A T S R G V L V V A A S G N S G A G S I S

E Q A V N S A T S R G V L V V A A S G N S G A G S I S

E Q A V N S A T S R G V L V V A A S G N S G A D S I S

E Q A V N S A T S R G V L V V A A S G N S G A G S I S

E Q A V N S A T S R G V L V V A A S G N S G A G S I S

E L A V N R A N N A G I L L V G A A G N T G R Q G V

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Y P A R Y A N A M A V G A T D Q N N N R A S F S Q Y G A G

A P A S Y A N A M A V G A T D Q N N N R A S F S Q Y G P G

Y P A R Y A N A M A V G A T D Q N N N R A S F S Q Y G A G

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Y P A R Y A N A M A V G A T D Q N N N R A S F S Q Y G A G

Y P A R Y A N A M A V G A T D Q N N N R A S F S Q Y G A G

N Y P A R Y S G V M A V A A V D Q N G Q R A S F S T Y G P E

Y P A R Y A N A M A V G A T D Q N N N R A S F S Q Y G A G

G Y P A K Y D S V I A V G A V D S N S N R A S F S S V G A E

G Y P G K Y P S V I A V G A V D S S N Q R A S F S S V G P E

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L D I V A P G V N V Q S T Y P G S T Y A S L N G T S M A T P

L D I V A P G V N V Q S T Y P G S T Y A S L N G T S M A T P

L D I V A P G V N V Q S T Y P G S T Y A S L N G T S M A T P

L D I V A P G V N V Q S T Y P G S T Y A S L N G T S M A T P

L D I V A P G V N V Q S T Y P G S T Y A S L N G T S M A T P

L D I V A P G V N V Q S T Y P G S T Y A S L N G T S M A T P

I E I S A P G V N V N S T Y T G N R Y V S L S G T S M A T P

L D I M A P G V N I Q S T Y P G S T Y A S D N G T S M A T P

L E V M A P G A G V Y S T Y P T N T Y A T L N G T S M A S P

L D V M A P G V S I Q S T L P G N K Y G A Y N G T S M A S P

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H V A G A A A L V L Q K N P S W S N V Q I R N H L K N T A T

H V A G A A A L V K Q K N P S W S N V Q I R N H L K N T A T

H V A G A A A L V K Q K N P S W S N V Q I R N H L K N T A T

H V A G A A V L V K H K N P S W S N V R I R D H L K K T A T

H V A G A A A L V K Q K N P S W S N V Q I R N H L K N T A T

H V A G A A A L V K Q K N P S W S N V Q I R N H L K N T A T

H V A G V A A L V K S R Y P S Y T N N Q I R Q R I N Q T A T

H V A G A A A L V K Q K N P S W S N V Q I R N H L K N T A T

H V A G A A A L I L S K H P N L S A S Q V R N R L S S T A T

H V A G A A A L I L S K H P N W T N T Q V R S S L Q N T T T

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K I S G T G T N F K Y G K I N S N K A V R Y

S L G S T N L Y G S G L V N A E A A T R

S L G S T N L Y G S G L V N A E A A T R

S L G S T N L Y G S G L V N A E A A T R

S L G S T N L Y G S G L V N A E A A T R

S L G S T N L Y G S G L V N A E A A T R

S L G S T N L Y G S G L V N A E A A T R

S L G S T N L Y G S G L V N A E A A A R

Y L G S P S L Y G N G L V H A G R A T Q

S L G S T N L Y G S G L V N A E A A T R

Y L G S S F Y Y G K G L I N V E A A A Q

K L G D S F Y Y G K G L I N V Q A A A Q

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Examples

Example 1

5

Identification of epitope sequences and epitope patterns.

High diversity libraries (10^{12}) of phages expressing random hexa-, nona- or dodecapetides as part of their membrane proteins, were screened for their capacity to bind purified specific rabbit IgG, and purified rat and mouse IgG1 and IgE antibodies. The phage libraries were obtained according to prior art (se WO 9215679 hereby incorporated by reference).

15 The antibodies were raised in the respective animals by subcutaneous, intradermal, or intratracheal injection of relevant proteins (e.g. proteases, lipolytic enzymes, amylases, oxidoreductases) dissolved in phosphate buffered saline (PBS). The respective antibodies were purified from the serum of immunised animals by affinity chromatography using paramagnetic immunobeads (Dynal AS) loaded with pig anti-rabbit IgG, mouse anti-rat IgG1 or IgE, or rat anti-mouse IgG1 or IgE antibodies.

25 The respective phage libraries were incubated with the IgG, IgG1 and IgE antibody coated beads. Phages, which express oligopeptides with affinity for rabbit IgG, or rat or mouse IgG1 or IgE antibodies, were collected by exposing these paramagnetic beads to a magnetic field. The collected phages were eluted from the immobilised antibodies by mild acid treatment, or by elution with intact enzyme. The isolated phages were amplified as known to the specialist. Alternatively, immobilised phages were directly incubated with E.coli for infection. In short, F-factor positive E.coli (e.g. XL-1 Blue, JM101, TG1) were infected with

M13-derived vector in the presence of a helper-phage (e.g. M13K07), and incubated, typically in 2xYT containing glucose or IPTG, and appropriate antibiotics for selection. Finally, cells were removed by centrifugation. This cycle of events was repeated 2-5 times on the respective cell supernatants. After selection round 2, 3, 4, and 5, a fraction of the infected E.coli was incubated on selective 2xYT agar plates, and the specificity of the emerging phages was assessed immunologically. Thus, phages were transferred to a nitrocellulose (NC) membrane. For each plate, 2 NC-replicas were made. One replica was incubated with the selection antibodies, the other replica was incubated with the selection antibodies and the immunogen used to obtain the antibodies as competitor. Those plaques that were absent in the presence of immunogen, were considered specific, and were amplified according to the procedure described above.

The specific phage-clones were isolated from the cell supernatant by centrifugation in the presence of polyethylenglycol. DNA was isolated, the DNA sequence coding for the oligopeptide was amplified by PCR, and the DNA sequence was determined, all according to standard procedures. The amino acid sequence of the corresponding oligopeptide was deduced from the DNA sequence.

Thus, a number of peptide sequences with specificity for the protein specific antibodies, described above, were obtained. These sequences were collected in a database, and analysed by sequence alignment to identify epitope patterns. For this sequence alignment, conservative substitutions (e.g. aspartate for glutamate, lysine for arginine, serine for threonine) were considered as one. This showed that most sequences were specific for the protein the antibodies were raised against. However, several cross-reacting sequences were obtained from phages that went through 2 selection rounds only. In the first round 22 epitope patterns were identified.

In further rounds of phage display, more antibody binding sequences were obtained leading to more epitope patterns. Further, the literature was searched for peptide sequences that have been
5 found to bind environmental allergen-specific antibodies (J All Clin Immunol 93 (1994) pp. 34-43; Int Arch Appl Immunol 103 (1994) pp. 357-364; Clin Exp Allergy 24 (1994) pp. 250-256; Mol Immunol 29 (1992) pp. 1383-1389; J Immunol 121 (1989) pp. 275-280; J. Immunol 147 (1991) pp. 205-211; Mol Immunol 29 (1992)
10 pp. 739-749; Mol Immunol 30 (1993) pp. 1511-1518; Mol Immunol 28 (1991) pp. 1225-1232; J. Immunol 151 (1993) pp. 7206-7213). These antibody binding peptide sequences were included in the database.

15 A first generation database of antibody binding peptides identified and their corresponding epitope patterns are shown in Table 2-7 below.

Tables 2-7: Overview of the antibody binding peptide sequences,
20 epitope patterns and epitope sequences. The type of antibody used for identifying the antibody binding sequences is indicated as IgG or IgE and the species from which the antibodies were derived are indicated as mo (mouse), ra (rat) and hu (human).

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Table 2: Savinase antibody binding peptide sequences, epitope patterns and epitope sequences.

Antibody binding peptide	Method of identification	Epitope pattern	Donor	Accession	Epitope sequence (BPN)	Epitope #	Ref
QVYVGDTS	Phage display	Q > Y > D >	savinase	savinase	Q208 V81 Y214 G80 D41 T208	sav1.1	Ra
LCVGS	Protein fragments		α -amylase inhibitor	savinase	L21 Q236 V26 Q25 S24	sav19.1	Hu
KRFANTELA	Phage display	R/K R F > N	savinase	savinase	K251 R247 A174 N173	sav6.1	Re-Mo
LDQIFTRW	Phage display	D/E Q I F F T	savinase	savinase	L42L75 D41 Q2 I79	sav5.1	Ra
FNDAPFKM	Phage display		savinase	savinase	N185 D181 A187 F189 V203	sav11.0	Ra
ANIPWRSRA	Phage display	> R S A	savinase	savinase	R145 S144 A142	sav3.2-lac1.0-lp4.0-pd5.0	Ra
ANIPWRSRA	Phage display	> R S A	savinase	savinase	S188 R186 S190 A179	sav3.1-lac1.0-lp4.0-pd5.0	Ra
RQSTDFGTT	Phage display	R Q > > D/E	savinase	savinase	R186 Q191 S156	sav2.2	Ra
VQVYVGDTS	Phage display	Q > Y > D >	savinase	savinase	Q191 Y192 G193/A194/G195 D197 S265	sav1.2	Ra
RFRSNATRA	Phage display	R/K R F > N	savinase	savinase	K251 R247 A174 N173	sav6.1	Re-Mo
CTARLRAGNACG	Phage display	AR > A	savinase	savinase	A172A169 R170 A194 G193 N261	sav10.4	Ra
LDQIFTRW	Phage display	D/E Q I F F T	savinase	savinase	D60 Q59 I44/I35	sav5.2	Ra
LDQIFTRW	Phage display	D/E Q I F F T	savinase	savinase	L42L75 D41 Q2 I79	sav5.1	Ra
EOIFFTSGL	Phage display	D/E Q I F F T	savinase	savinase	E112 Q109 I79	sav5.4	Ra
GRFSNSKFK	Phage display	L > G R S	savinase	savinase	L198 G195 R170 S163	sav6.2-lac1.0-lp5.1-5.2	Ra
AVLRDC	Protein fragments		α -amylase inhibitor	savinase	A254 V288 L267 R10 D181	sav18.1-pd18.1-18.2	Hu
LCVGS	Protein fragments		α -amylase inhibitor	savinase	L217 Q206 V81 G80 S3	sav19.2	Hu
LRQCNRCV	Phage display	R Q > > D/E	savinase	savinase	L267 R10 Q12 N269 E271 R275	sav2.1	Ra
SPVTKRASLKIDSKK	Protein fragments		Der p II	savinase	A88 S87/T22 L233 K235 I246	sav16.0-pd7.0	Hu
RQSTDFGTT	Phage display	R Q > > D/E	savinase	savinase	R247 Q245 S240/S242	sav2.3	Ra
FCTNNCELS	Phage display	N > > E L	savinase	savinase	T143 N173 N140 E136 L135	sav7.2	Ra
FCTNNCELS	Phage display	N > > E L	savinase	savinase	N117 N116 E112 L111	sav7.1	Ra
DFHVKYAAQ	Phage display		savinase	savinase		sav6.0	Ra
VAGTKALPVLENA	Protein fragments		Fel d I	savinase	L135 P168 V139 L111 E112 N116	sav12.0-pd8.0	Hu
AAYPDV	Protein fragments	A > > > Y P >	α -amylase inhibitor	savinase	A215 Y214 P40 D41 V81	sav13.0-pd13.1-13.2	Hu
EQIFFTSGL	Phage display	D/E Q I F F T	savinase	savinase	E271 Q12 I8	sav5.3	Ra
VDAAF	Protein fragments		Poa d IX	savinase	V203 D181 A179 A187 F189	sav15.0-pd12.0	Hu
IRAFRRNANW	Protein fragments		α -amylase inhibitor	savinase	A232 V234 L250 R247 D197	sav18.2-pd18.1-18.2	Hu
CTARLRAGNACG	Phage display	AR > A	savinase	savinase	A272/A273 R275 R19 N18 A15/A16	sav10.1	Ra
TFHDAPALQ	Phage display	AR > A	savinase	savinase	A15/A16 R19 L21 R275 A272 A273 N269	sav10.2	Ra
CTARWALGVCG	Phage display	AR > A	savinase	savinase	H39 D41 A74/A73 P86 A88 L90	sav4.0	Ra
GRFSNSKFK	Phage display	L > G R S	savinase	savinase	R145 G146 R145 S144/S141 N140	sav10.3	Ra
IRFANDHTR	Phage display	R/K R F > N	savinase	savinase	K27 R45 N43 D41 H39 T38/T213	sav9.1-lac1.0-lp5.1-5.2	Ra
KRFANTEPA	Phage display	R/K R F > N	savinase	savinase	K251 R247 A174 N173	sav6.2	Re-Mo

YKVSAL TKKVS	Protein fragments	Protein fragments	a-amylase inhibitor	savinase	Y91 K27 V26 S24 G23 L21 S24 G25 K27 Y91 V93	sav14.0-pd14.0 sav17.0-pd17.1-17.2	Hu Hu

Table 3: PD498 antibody binding peptide sequences, epitope patterns and epitope sequences.

Epitope pattern	Accession	Epitope Sequence (BPN)	Epitope	Host
A>>>Y P>	pd498	V198 A254 Q252 Y276 K239 A235 L233 P86	pd8.0	Hu
>KL>>	pd498	*3aA Y1Y2 P4P1 D-2 V81	pd13.2	Hu
>KL>>	pd498	S182 Y6 G7 P8 T13 P14 A15 A16	pd11.0	Hu
>KL>>	pd498	Y171 K136 L135 A108 Y113	pd4.4	Hu
>KL>>	pd498	Y48/Y37 K46 *44aaV A43 L42	pd14.0	Hu
KQS	pd498	V198V198 D197 A174/A176 A188 F163	pd12.0	Hu
KQS	pd498	A142 A147 V148 K120 Q27 S24/S25	pd2.3	Hu
>KL>>	pd498	R44 K89 Q27 S236 K120 G146	pd2.2	Ra
>KL>>	pd498	*28aV T88 *44a K R44 A43 L42	pd7.0	Hu
>KL>>	pd498	N59/N55 K48 L91 A29/A119 T28	pd4.3	Ra
>KL>>	pd498	N240/N243 K239 L233/L234 A16 T21 R22	pd4.1	Ra
>KL>>	pd498	Y37 K48 L91 A114 Y113	pd4.5	Hu
>KL>>	pd498	N240/N243 K239 L233/L234 A16 T21 R22	pd4.1	Ra
Y>KL	pd498	Y113 I111 A108/A138 K136 L135	pd3.1	Ra
KQS	pd498	A115 K145 N243 N240 K239 Q237 S236	pd2.1	Ra
>RY>K/R	pd498	R94 R53 Y48 Q117 R112 S109/S137	pd1.5-lac2.0	Ra
>RY>K/R	pd498	A168 Q167 F163 T182 S160 G193	pd10.0	Hu
>RY>K/R	pd498	Y276 I246 K239 L234 S236	pd3.2	Ra
>RY>K/R	pd498	N240/N243 K239 L233/L234 R22 P86	pd4.2	Ra
>RY>K/R	pd498	*3aA Y2 P14 D18 V19	pd13.1	Hu
KQS	pd498	A15 A16 V274 K239 Q237 S236	pd2.4	Hu
>RY>K/R	pd498	G148 K145 Y141 V139 S137	pd17.2	Hu
>RY>K/R	pd498	A273 V274 L233 R22 D87	pd18.1	Hu
AR>A	pd498	N10 S12 A15/A16 R275 A273/A249 R247 A174	pd9.0	Hu+Ra
>RY>K/R	pd498	D197 S170	pd6.2	Ra
>RY>K/R	pd498	R22 G23 L233 S236	pd1.4-lac2.0	Ra
>RY>K/R	pd498	R94 R53 Y48 P57 K46 L91	pd1.4-lac2.0	Ra
>RY>K/R	pd498	R94 R53 Y48 P57 K46 L91	pd15.0	Hu
>RY>K/R	pd498	L96 R94 S33 V35 Y37	pd1.3-lac2.0	Ra
>RY>K/R	pd498	S109/S137 R112 Y141 N144 K145	pd1.2-lac2.0	Ra
>RY>K/R	pd498	T162 R161 Y192 N191 K186	pd1.1-lac2.0	Ra
>RY>K/R	pd498	T133/T134 R112 Y141 N144 K145	pd18.2	Hu
>RY>K/R	pd498	A92 *44aaV L42 R44 D75	pd17.1	Hu
>RY>K/R	pd498	S236 G238 K239 Y276 V274 S270	pd16.0	Hu
>RY>K/R	pd498	S12 P14 W17 S-5 W-6	pd5.0-lac1.0-lp4.0-sav3.1-3.2	Ra
>RY>K/R	pd498	S137 R112 S109 A108	pd6.1	Ra
>RY>K/R	pd498	S215 M217 I205 M222 G219		

Table 4: Antibody binding peptide sequences, epitope patterns and epitope sequences for the *T. lanuginosus* lipase (Lipolase).

Antibody binding peptide	Peptide sequence	Epitope pattern	Epitope sequence	Epitope #	Host
QRPRRYELE	Phage display	R P P R	L124 E129 Y164	lip1.0	Ra
IELEVRPPRO	Phage display	> E Y	H215 E219 Y220	lip2.1	Ra
HEYDMRVAV	Phage display	> E Y	H215 E219 Y220	lip2.2	Ra
HEYMDVHL	Phage display	> E Y	S217 E219 Y220	lip2.3	Ra
SEYSMSITP	Phage display	> P > P A P > S	P253 P250 A243 P208/P207 S214/S216/S217	lip3.0	Ra
CVWPAHAPLSCG	Phage display	> P > P A P > S	P253 P250 A243 P208/P207 S214/S216/S217	lip3.0	Ra
CSWPSPAPLSCG	Phage display	> P > P A P > S	P253 P250 A243 P208/P207 S214/S216/S217	lip3.0	Ra
CDFFLHAPLSCG	Phage display	> P > P A P > S	P253 P250 A243 P208/P207 S214/S216/S217	lip3.0	Ra
CLFSPAPRSCG	Phage display	> P > P A P > S	P253 P250 A243 P208/P207 S214/S216/S217	lip3.0	Ra
CDGPAPAPWSCG	Phage display	> P > P A P > S	P253 P250 A243 P208/P207 S214/S216/S217	lip3.0	Ra
CSFPLPAPRSCG	Phage display	> P > P A P > S	P253 P250 A243 P208/P207 S214/S216/S217	lip3.0	Ra
CVYPSAPAPWSCG	Phage display	> P > P A P > S	P253 P250 A243 P208/P207 S214/S216/S217	lip3.0	Ra
PEYTMNALS	Phage display	> E Y	P218 E219 Y220	lip2.4	Ra
CSRSAGKARLCG	Phage display	> R S A	R209 S214 A182	lip4.0-lac1.0-pd5.0-sav3.1-3.2	Ra
LEYPMASQ	Phage display	> E Y	L124 E129 Y164	lip2.1	Ra
RKLTLSGRS	Phage display	L > G R S	L67 G65 R81 S63/S85	lip5.1-je4.0-sav9.0	Ra
RKLTLSGRS	Phage display	L > G R S	L67 G65 R81 S63/S85	lip5.2-je4.0-sav9.0	Ra
SYGAPATPAA	Protein fragments	Poa p IX	S170 Y171 G172 A173 P174 A150 T153	lip6.0	Ra
PAAGYTPAAP	Protein fragments	Poa p IX	A18/A19/A20 G65 Y53 T123	lip7.0	Hu
YKLAY	Protein fragments	Poa p IX	Y138 K74 L75 A69 Y16	lip8.1	Hu
YKLAY	Protein fragments	Poa p IX	Y53 K127 L67 A68 Y16	lip8.2	Hu
KYDDYVATLS	Protein fragments	Poa p IX	Y194 D167 D165 Y184 V132 A131 L52 S54	lip9.0	Hu
EYKATPAGEL	Protein fragments	Der p I	E43 V44 K46 A47 T72	lip10.0	Hu
CGYSNAQGVYWI	Protein fragments	Der p I	Y53 S54 N25/N26 A18/A19/A20 Q15 V44	lip15.0	Hu
VFGIDFNACHYMKC	Protein fragments	Der p I	P256 I255 D254 P253 N200 H198 Y261	lip16.0	Hu
SPVTKRASKLDSKK	Protein fragments	Ovalbumin	R179 A182 S216/S217 I238 K237 I235 D234 S224 K223	lip17.0	Hu
IMSALAMVYLGAKE	Protein fragments	a-amylase	V140 Y138 L69 A49 A47 K46	lip18.0	Hu
ELGVRE	Protein fragments	Inhibitor	E69 L67 G109/G177 V178 R175 D242	lip11.0	Hu
GCGRKEV	Protein fragments	a-amylase	G106 C107 R108 K98 E99	lip12.0	Hu

Table 5: Amylase (Natalase) antibody binding peptide sequences, epitope patterns and epitope sequences.

Antibody binding peptide	Method of identification	Epitope pattern	Donor	Acceptor	Epitope sequence	Epitope pattern	Epitope sequence
ARIDPRGFS	Phage display	A > I D P R/K	amy/lase	amy/lase	A380 K381 I382 D383 P384 R389	je1.1	Ra
ARIDPRHGS	Phage display	A > I D P R/K	amy/lase	amy/lase	A380 K381 I382 D383 P384 R389	je1.1	Ra
CSVAKIDPRITCG	Phage display	A > I D P R/K	amy/lase	amy/lase	A109 K138 D140 P142 R144	je1.2	Ra
CSVAKIDPRITCG	Phage display	A > I D P R/K	amy/lase	amy/lase	A380 K381 I382 D383 P384 R389	je1.1	Ra
AKIDPKPDT	Phage display	A > I D P R/K	amy/lase	amy/lase	A109 K138 D140 P142 R144	je1.2	Ra
AKIDPKPDT	Phage display	A > I D P R/K	amy/lase	amy/lase	A380 K381 I382 D383 P384 R389	je1.1	Ra
ARIDPRHGS	Phage display	A > I D P R/K	amy/lase	amy/lase	A109 K138 D140 P142 R144	je1.2	Ra
QIYNDTGPT	Phage display	Q > Y > D >	amy/lase	amy/lase	Q380 L386 Y388 Y387 D366	je2.4	Ra
QIYNDTGPT	Phage display	Q > Y > D >	amy/lase	amy/lase	Q170 I173 Y186 D195	je2.3	Ra
QIYNDTGPT	Phage display	Q > Y > D >	amy/lase	amy/lase	Q357 I352 Y349 D366	je2.2	Ra
QIYNDTGPT	Phage display	Q > Y > D >	amy/lase	amy/lase	Q331 I370 Y368 Y367 D366	je2.1	Ra
CGSATIDPRQCG	Phage display	A > I D P R/K	amy/lase	amy/lase	A109 K138 D140 P142 R144	je1.2	Ra
CNADNQMYPCQG	Phage display	A > > > Y P >	amy/lase	amy/lase	N29 A27 D26/D25 Y8 P41/P42	je3.1	Ra
ARIDPRGFS	Phage display	A > I D P R/K	amy/lase	amy/lase	A109 K138 D140 P142 R144	je1.2	Ra
CGSATIDPRQCG	Phage display	A > I D P R/K	amy/lase	amy/lase	A380 K381 I382 D383 P384 R389	je1.1	Ra
CPADSSGYPLCG	Phage display	A > > > Y P >	amy/lase	amy/lase	A107/A109 D108 Y57 P41/P42	je3.3	Ra
QLYGDEQLP	Phage display	Q > Y > D >	amy/lase	amy/lase	Q331 I370 Y368 Y367 D366	je2.1	Ra
QLYGDEQLP	Phage display	Q > Y > D >	amy/lase	amy/lase	Q357 I352 Y348 D366	je2.2	Ra
QLYGDEQLP	Phage display	Q > Y > D >	amy/lase	amy/lase	Q170 I173 Y186 D195	je2.3	Ra
QLYGDEQLP	Phage display	Q > Y > D >	amy/lase	amy/lase	Q390 L386 Y388 Y387 D366	je2.4	Ra
RYAQIDPRW	Phage display	A > I D P R/K	amy/lase	amy/lase	A380 K381 I382 D383 P384 R389	je1.1	Ra
RYAQIDPRW	Phage display	A > I D P R/K	amy/lase	amy/lase	A109 K138 D140 P142 R144	je1.2	Ra
GEFNLGRSS	Phage display	L > G R S	amy/lase	amy/lase	L88 G92 R31 S28	je4.1-sav9.0-1p5.1-5.2	Ra
CNADSWGYPKCG	Phage display	A > > > Y P >	amy/lase	amy/lase	N29 A27 D26/D25 Y8 P41/P42	je3.1	Ra
CNADNQMYPCQG	Phage display	A > > > Y P >	amy/lase	amy/lase	N102 A233 D232 Y54 P41/P42	je3.2	Ra
CNADSWGYPKCG	Phage display	A > > > Y P >	amy/lase	amy/lase	N102 A233 D232 Y54 P41/P42	je3.2	Ra
GEFNLGRSS	Phage display	L > G R S	amy/lase	amy/lase	L62 G63/G76 R78 S79	je4.2-sav9.0-1p5.1-5.2	Ra

Table 6: Cellulase (Carezyme; Cel45 from Humicola insolens) antibody binding peptide sequences, epitope patterns and epitope sequences.

Antibody binding peptide	Method of identification	Epitope pattern	Donor	Acceptor	Epitope sequence	Epitope	IdG
CVHAGPRAGTCG	Phage display	> G > A G	Carezyme	Carezyme	P23 R201 A83 G84	car1.1	Ra
CVHAGPRAGTCG	Phage display	VH > G >	Carezyme	Carezyme		car2.0	Ra
CLSGPLAGRVCG	Phage display	> G > A G	Carezyme	Carezyme	P23 R201 A83 G84	car1.1	Ra
CRISPWYSVPCG	Phage display		Carezyme	Carezyme		car3.0	Ra
CLSGPAAGQSCG	Phage display	> G > A G	Carezyme	Carezyme	P23 R201 A83 G84	car1.1	Ra
CITRGTRAGWCG	Phage display	A > D P R/K	le-1	Carezyme	R146 I131 D133 P137	car11.2	Ra
CITRGTRAGWCG	Phage display	> G > A G	Carezyme	Carezyme	P23 R201 A83 G84	car1.1	Ra
CLSGPAAGQSCG	Phage display	AR > A	savinase	Carezyme	A191 R200 R201 A83 N81	car6.2	Ra
CLSGPAAGQSCG	Phage display	> G > A G	Carezyme	Carezyme	T95/S96 G27 P98 A100 G101	car1.2	Ra
CLSGPAAGQSCG	Phage display	A > D P R/K	le-1	Carezyme	A195 R37 I38 D40 L44	car11.1	Ra
CLSGPAAGQSCG	Phage display	Q > Y > D >	savinase, le-1	Carezyme	Q59 Y54 G134 D133 T136	car10.0	Ra
CITRGTRAGWCG	Phage display	> P > A P > S	lipoptime	Carezyme	W62W169 P61 P165 A162 P160	car8.0	Ra
CITRGTRAGWCG	Phage display	> G > A G	Carezyme	Carezyme	T95/S96 G27 P98 A100 G101	car1.2	Ra
CLSGPLAGRVCG	Phage display	R/K R F > N	savinase	Carezyme	R7 R170 F174 A177	car7.0	Ra
CLSGPLAGRVCG	Phage display	> G > A G	Carezyme	Carezyme	T95/S96 G27 P98 A100 G101	car1.2	Ra
CLSGPLAGRVCG	Phage display	AR > A	savinase	Carezyme	A1 R4 R7 A177 N176	car6.1	Ra
CLSGPLAGRVCG	Phage display	> P > R D T G	laccase	Carezyme	D178 P180 R4 D2 S183	car5.0	Ra
CLSGPLAGRVCG	Phage display	> R Y > K/R	pd498	Carezyme	R170 R153 Y168 P165 K164 L163	car4.0	Ra
CLTAGPSAGYCG	Phage display	D/E Q I F T	savinase	Carezyme	Q36 I38 F41 F29 T197	car8.0	Ra
CYTTRLAGLCLG	Phage display	> G > A G	Carezyme	Carezyme	T95/S96 G27 P98 A100 G101	car1.2	Ra
CYTTRLAGLCLG	Phage display	> G > A G	Carezyme	Carezyme	P23 R201 A83 G84	car1.1	Ra
CVHSGPRAGYCG	Phage display	> G > A G	Carezyme	Carezyme	T95/S96 G27 P98 A100 G101	car1.2	Ra
CVHSGPRAGYCG	Phage display	> G > A G	Carezyme	Carezyme	P23 R201 A83 G84	car1.1	Ra
CVHSGPRAGYCG	Phage display	VH > G >	Carezyme	Carezyme		car2.0	Ra
CVHSGPRAGYCG	Phage display	> G > A G	Carezyme	Carezyme	T95/S96 G27 P98 A100 G101	car1.2	Ra
CVHSGPRAGYCG	Phage display	> G > A G	Carezyme	Carezyme	T95/S96 G27 P98 A100 G101	car1.2	Ra
CVHSGPRAGYCG	Phage display	VH > G >	Carezyme	Carezyme		car2.0	Ra
CVTRGNAGSCG	Phage display	> G > A G	Carezyme	Carezyme	T95/S96 G27 P98 A100 G101	car1.2	Ra
CVTRGNAGSCG	Phage display	> G > A G	Carezyme	Carezyme	P23 R201 A83 G84	car1.1	Ra
CVTRGNAGSCG	Phage display	> G > A G	Carezyme	Carezyme	P23 R201 A83 G84	car1.1	Ra

CITSGPRAGNCG	Phage display	> G >> A G	carezyme	T95/S96 G27 P98 A100 G101	carl.2	Ra
CITSGPRAGNCG	Phage display	> G >> A G	carezyme	P23 R201 A83 G84	carl.1	Ra

Table 7: Laccase (*Myceliophthora thermophila* laccase) antibody binding peptide sequences, epitope patterns and epitope sequences.

Antibody binding inhibitor	Method of identification	Epitope pattern	Donor	Antibody	Epitope sequence	Epitope	IdS	IgG
POSDPGESQ	Phage display	P > S/T D P G	laccase	laccase	P180 R175 T168 D166 P165 G265	lac3.2	Ra	Ra
WPKSDAGDS	Phage display	P > > D A G	laccase	laccase	P241 R409 S410/S416 D434 A389 G390	lac4.1	Ra	Ra
POSADGWM	Phage display	P > > D A G	laccase	laccase	P241 R409 S410/S416 D434 A389 G390	lac4.1	Ra	Ra
DPVRDTGAG	Phage display	P > R D T G	laccase	laccase	P241 R409 D434 T432 G430/G390	lac5.1	Ra	Ra
GPSRDAGLL	Phage display	P > > D A G	laccase	laccase	P241 R409 S410/S416 D434 A389 G390	lac4.1	Ra	Ra
PASDAGRGP	Phage display	P > > D A G	laccase	laccase	P241 R409 S410/S416 D434 A389 G390	lac4.1	Ra	Ra
PRDSTGLAL	Phage display	P > S/T D P G	laccase	laccase	P378 R379 T442 D443 P445 G446	lac3.1	Ra	Ra
POSDPGESQ	Phage display	P > S/T D P G	laccase	laccase	P378 R379 T442 D443 P445 G446	lac3.1	Ra	Ra
RYPLRATN	Phage display	> R Y > K/R	laccase	laccase		lac2.0- pd1.1-1.4	Ra	Ra
GAARDARSA	Phage display	> R S A	laccase	laccase		lac1.0- lp4.0-pd5.0- sav3.1-3.2	Ra	Ra
PRSDTGFGS	Phage display	P > R D T G	laccase	laccase	P241 R409 D434 T432 G430/G390	lac5.1	Ra	Ra
LPRSDPGGR	Phage display	P > S/T D P G	laccase	laccase	P180 R175 T168 D166 P165 G265	lac3.2	Ra	Ra
DPARDTGDV	Phage display	P > R D T G	laccase	laccase	P241 R409 D434 T432 G430/G390	lac5.1	Ra	Ra
APKSDNGIT	Phage display	P > > D A G	laccase	laccase	P241 R409 S410/S416 D434 A389 G390	lac4.1	Ra	Ra
PKSDPGTNW	Phage display	P > S/T D P G	laccase	laccase	P378 R379 T442 D443 P445 G446	lac3.1	Ra	Ra
PRIDPGWLA	Phage display	P > S/T D P G	laccase	laccase	P378 R379 T442 D443 P445 G446	lac3.1	Ra	Ra
LPRSDPGGR	Phage display	P > S/T D P G	laccase	laccase	P378 R379 T442 D443 P445 G446	lac3.1	Ra	Ra
PSSDPGARS	Phage display	P > S/T D P G	laccase	laccase	P180 R175 T168 D166 P165 G265	lac3.2	Ra	Ra
HVFDKNVTR	Phage display		laccase	laccase		lac6.0		
PRSDPGTPT	Phage display	P > S/T D P G	laccase	laccase	P378 R379 T442 D443 P445 G446	lac3.1	Ra	Ra
PRSDPGTPT	Phage display	P > S/T D P G	laccase	laccase	P180 R175 T168 D166 P165 G265	lac3.2	Ra	Ra

PRDSTGLAL	Phage display	P > S/T D P G	laccase	P160 R175 T168 D166 P165 G265	lac3.2	Ra
PRTPDGLA	Phage display	P > S/T D P G	laccase	P160 R175 T168 D166 P165 G265	lac3.2	Ra
PSSDPGARS	Phage display	P > S/T D P G	laccase	P378 R379 T442 D443 P445 G446	lac3.1	Ra
PKSPDGTNW	Phage display	P > S/T D P G	laccase	P160 R175 T168 D166 P165 G265	lac3.2	Ra
WPKSDAGDS	Phage display	P > > D A G	laccase	P350 S349 D80 A79 G78	lac4.2	Ra
QPSDAGVWM	Phage display	P > > D A G	laccase	P350 S349 D80 A79 G78	lac4.2	Ra
GPSRDAGLL	Phage display	P > > D A G	laccase	P350 S349 D80 A79 G78	lac4.2	Ra
PASDAGRGP	Phage display	P > > D A G	laccase	P350 S349 D80 A79 G78	lac4.2	Ra
APKSDNGIT	Phage display	P > > D A G	laccase	P300 R234 S211 D213 A286	lac4.2	Ra
WPKSDAGDS	Phage display	P > > D A G	laccase	P300 R234 S211 D213 A286	lac4.3	Ra
QPSDAGVWM	Phage display	P > > D A G	laccase	P300 R234 S211 D213 A286	lac4.3	Ra
GPSRDAGLL	Phage display	P > > D A G	laccase	P300 R234 S211 D213 A286	lac4.3	Ra
PASDAGRGP	Phage display	P > > D A G	laccase	P300 R234 S211 D213 A286	lac4.3	Ra
APKSDNGIT	Phage display	P > > D A G	laccase	P300 R234 S211 D213 A286	lac4.3	Ra
DPVRODTGAG	Phage display	P > > R D T G	laccase	P378 R379 D469 T473 G446	lac5.2	Ra
PRSDTGFGS	Phage display	P > > R D T G	laccase	P378 R379 D469 T473 G446	lac5.2	Ra
DPARDTGDV	Phage display	P > > R D T G	laccase	P378 R379 D469 T473 G446	lac5.2	Ra
DPVRODTGAG	Phage display	P > > R D T G	laccase	P60 R59 D51/D53 T10/T12 G30	lac5.3	Ra
PRSDTGFGS	Phage display	P > > R D T G	laccase	P60 R59 D51/D53 T10/T12 G30	lac5.3	Ra
DPARDTGDV	Phage display	P > > R D T G	laccase	P60 R59 D51/D53 T10/T12 G30	lac5.3	Ra
DPVRODTGAG	Phage display	P > > R D T G	laccase	P157/P155 R23 D118 T114 G113	lac5.4	Ra
PRSDTGFGS	Phage display	P > > R D T G	laccase	P157/P155 R23 D118 T114 G113	lac5.4	Ra
DPARDTGDV	Phage display	P > > R D T G	laccase	P157/P155 R23 D118 T114 G113	lac5.4	Ra

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Example 2

Localisation of epitope sequences and epitope areas on the 3D-structure of acceptor proteins.

5

Epitope sequences were assessed manually on the screen on the 3D-structure of the protein of interest, using appropriate software (e.g. SwissProt Pdb Viewer, WebLite Viewer).

- 10 In a first step, the identified epitope patterns were fitted with the 3D-structure of the enzymes. A sequence of at least 3 amino acids, defining a specific epitope pattern, was localised on the 3D-structure of the acceptor protein. Conservative mutations (e.g. aspartate for glutamate, lysine for arginine, serine
15 for threonine) were considered as one for those patterns for which phage display had evidenced such exchanges to occur. Among the possible sequences provided by the protein structure, only those were retained where the sequence matched a primary sequence, or where it matched a structural sequence of amino acids,
20 ids, where each amino acid was situated within a distance of 5Å from the next one. Occasionally, the mobility of the amino acid side chains, as provided by the software programme, had to be taken in to consideration for this criterium to be fulfilled.
- 25 Secondly, the remaining anchor amino acids as well as the variable amino acids, i.e. amino acids that were not defining a pattern but were present in the individual sequences identified by phage library screening, were assessed in the area around the various amino acid sequences localised in step 1. Only amino acids
30 ids situated within a distance of 5Å from the next one were included.

Finally, an accessibility criterium was introduced. The criterium was that at least half of the anchor amino acids had a sur-

face that was >30% accessible. Typically, 0-2 epitopes were retained for each epitope pattern. In some cases, two different amino acids could with equal probability be part of the epitope (e.g. two leucines located close to each other in the protein 3D-structure). For example, in Savinase two epitopes actually fit to the antibody binding peptide LDQIFFTRW: L75 D41 Q2 I79 and L42 D41 Q2 I79. A shorthand notation for such a situation is: L42/L75 D41 Q2 I79.

Thus, a number of epitope sequences were identified and localised on the surface of various proteins. As suggested by sequence alignment of the antibody binding peptides, structural analysis confirmed most of the epitopes to be enzyme specific, with only few exceptions. Overall, most of the identified epitopes were at least partially structural. However, some proteins (e.g. amylase) expressed predominantly primary sequence epitopes. Typically, the epitopes were localised in very discrete areas of the enzymes, and different epitope sequences often shared some amino acids (hot-spots).

20

The identified epitope sequences are shown in Tables 2-7.

Birch allergen:

Bet v1 (W099/47680) was used as the parent protein for identification of epitope sequences that may cross react with enzyme epitopes. The structural coordinates from 1BV1.pdb (Gajhede et al., NAT.STRUCT.BIOL., Vol. 3, p. 1040, 1996) were used as well the corresponding sequence (Swissprot accession number P15494). The epitope pattern P>PAP>S (which had been identified from antibody binding peptides specific for anti-Lipolase antibodies) was found to match three (overlapping) epitope sequences on the surface of Bet v1:

Bet v1 1.1: P31 A34 P35 A37 P59 S39/S40;

Bet v1 1.2: P63 L62 P59 A37 P35 S39/S40; and

Bet v1 1.3: P59 S39/S40 P31 A34 P35 S39/S40.

5 Example 3

Epitope areas

It is common knowledge that amino acids that surround binding
 10 sequences can affect binding of a ligand without participating
 actively in the binding process. Based on this knowledge, areas
 covered by amino acids with potential steric effects on the epi-
 tope-antibody interaction, were defined around the identified
 epitopes. Practically, all amino acids situated within 5Å from
 15 the amino acids defining the epitope were included. The accessi-
 bility criterium was not included for defining epitope areas, as
 hidden amino acids can have an effect on the surrounding struc-
 tures.

20 For Savinase, the following amino acid residues belong to the
 epitope area that correspond to each epitope sequence indicated
 in Table 2:

25	sav1.1	A1	Q2	S3	P5	H39	P40	D41	L42	N43
	G63	T66	H67	A69	G70	T71	A73	A74	L75	N77
	S78	I79	G80	V81	L82	G83	N204	V205	Q206	S207
	T208	Y209	P210	S212	T213	Y214	A215	S216	L217	
30	sav1.2	S153	G154	N155	S156	G157	A158	G160	S161	I162
	S163	A169	R170	A174	M175	A176	V177	G178	R186	F189
	S190	Q191	Y192	G193	A194	G195	L196	D197	I198	V199
	T220	R247	K251	A254	T255	S256	T260	N261	L262	Y263
	G264	S265	G266	L267						

156

sav2.1	W6	G7	I8	R10	V11	Q12	A13	P14	A15
	A16	R19	L21	V84	T180	D181	Q182	N183	N184 I198
	V199	A200	P201	H226	V227	A230	L233	V234	K237 N238
	H249	L250	T253	A254	T255	S256	L257	S265	G266 L267
5	V268	N269	A270	E271	A272	A273	T274	R275	
sav2.2	S153	G154	N155	S156	G157	A158	S161	I162	S163
	G178	A179	T180	D181	N184	N185	R186	A187	S188 F189
	S190	Q191	Y192	G193	L196	T220	L262	Y263	
10									
sav2.3	A142	T143	G146	V147	L148	Y171	A172	N173	A174
	M175	D197	A231	V234	K235	N238	P239	S240	W241 S242
	N243	V244	Q245	I246	R247	N248	H249	L250	K251
15									
sav3.1	S153	G154	N155	S156	G157	A158	V177	G178	A179
	T180	D181	N184	N185	R186	A187	S188	F189	S190 Q191
	Y192	V199	A200	P201	G202	V203	N218	G219	T220 A223
20	L262	Y263							
sav3.2	L111	E112	G115	N116	M119	A138	V139	N140	S141
	A142	S144	R145	G146	V147	V149	N173	N243	
25									
sav4.0	Q2	H17	T22	G23	S24	G25	V26	K27	V28
	V30	I35	S37	T38	H39	P40	D41	L42	N43 I44
	R45	G46	T66	A69	G70	T71	I72	A73	A74 L75
	N76	N77	I79	G80	V81	L82	G83	V84	A85 P86
30	S87	A88	E89	L90	Y91	A92	T208	Y209	P210 S212
	T213	Y214							
sav5.1	A1	Q2	S3	V4	I35	S37	H39	P40	D41
	L42	N43	I44	T66	A69	G70	A73	A74	L75 N76

157

	N77	S78	I79	G80	V81	L82	G83	P86	L90	T208
	Y214									
	sav5.2	V30	T33	G34	I35	S37	T38	L42	N43	I44
5	R45	G46	E54	S57	T58	Q59	D60	G61	N62	G63
	H64	G65	T66	H67	A69	L90	Y91	A92	K94	P210
	sav5.3	V4	P5	W6	G7	I8	S9	R10	V11	Q12
10	A13	P14	A15	A16	R19	N269	A270	E271	A272	A273
	T274	R275								
	sav5.4	A1	Q2	P40	D41	F50	L75	N77	S78	I79
	G80	V81	V104	S105	S106	I107	A108	Q109	G110	L111
15	E112	W113	A114	G115	N116	Q137	A138	S141	A142	Y214
	sav6.1	V139	N140	T143	L148	V149	A151	P168	A169	Y171
	A172	N173	A174	M175	A176	D197	I198	N243	V244	Q245
20	I246	R247	N248	H249	L250	K251	N252	T253	A254	S265
	sav6.2	Q2	G25	V26	K27	V28	A29	I35	S37	T38
	H39	P40	D41	L42	N43	I44	R45	G46	G47	Q59
25	T66	A69	G70	A73	A74	L75	N77	I79	G80	V81
	L82	A88	E89	L90	Y91	N117	G118	M119	H120	V121
	S207	T208	Y209	P210	G211	S212	T213	Y214	A215	
	sav7.1	K27	L31	I107	A108	Q109	G110	L111	E112	W113
30	A114	G115	N116	N117	G118	M119	A122	L124	L135	Q137
	A138	V139	S141	A142	R145	V149				
	sav7.2	V104	I107	A108	L111	S132	A133	T134	L135	E136
	Q137	A138	V139	N140	S141	A142	T143	S144	R145	G146

158

V147 V149 Y167 P168 Y171 A172 N173 A174 M175 N243
R247

sav9.1 L111 E112 A114 G115 N116 M119 H120 V121 A122
5 E136 Q137 A138 V139 N140 S141 A142 T143 S144 R145
G146 V147 L148 V149 V150 N173 M175 N243 I246 R247
L250

sav9.2 L126 G127 S128 P129 A152 S153 G154 S161 I162
10 S163 Y167 P168 A169 R170 Y171 A172 A176 V177 G178
Q191 Y192 G193 A194 G195 L196 D197 I198 V199 T260
N261 L262 Y263 G264

sav10.1 Q12 A13 P14 A15 A16 H17 N18 R19 G20
15 L21 T22 N76 L82 G83 V84 A85 P86 L233 V234
K237 N238 H249 L250 T253 N269 A270 E271 A272 A273
T274 R275

sav10.2 V11 Q12 A13 P14 A15 A16 H17 N18 R19
20 G20 L21 T22 G23 L233 V234 Q236 K237 N238 H249
L250 T253 A254 T255 L267 V268 N269 A270 E271 A272
A273 T274 R275

sav10.3 L31 D32 H64 V68 V95 L96 I107 L111 A114
25 G115 N116 M119 V121 A122 N123 L124 S125 L126 G127
S128 P129 V139 S141 A142 T143 S144 R145 G146 V147
L148 V149 V150 A151 A152 S153 S163 Y167 P168 A169
N173 A174 M175 A176 V177 T220 S221 M222 T224 P225
V227 A228 A231 N243 I246 R247 L250

30 sav10.4 P131 S132 A133 L135 E136 V139 A151 A152 S153
G160 S161 I162 S163 Y167 P168 A169 R170 Y171 A172
N173 A174 A176 Q191 Y192 G193 A194 G195 L196 R247

160

	sav17.0	T22	G23	S24	G25	V26	K27	V28	A29	V30
	L31	D32	I35	I44	R45	G46	G47	A48	F50	S87
	A88	E89	L90	Y91	A92	V93	K94	V95	G110	W113
	N117	G118	M119	H120	V121	A232	K235	Q236		
5	sav18.1	W6	G7	I8	S9	R10	V11	Q12	A179	T180
	D181	Q182	N183	N184	N185	R186	A187	I198	V199	A200
	P201	V203	H226	V227	A230	H249	L250	K251	N252	T253
	A254	T255	S256	L257	S265	G266	L267	V268	N269	A270
10	sav18.2	A13	A16	H17	L21	T22	G23	V26	V28	V84
	A85	A88	V121	L148	Y171	A172	N173	V174	M175	A176
	G195	L196	D197	I198	V199	V227	A228	G229	A230	A231
15	A232	L233	V234	K235	Q236	K237	N238	W241	N243	V244
	Q245	I246	R247	N248	H249	L250	K251	N252	T253	A254
	Y263	G264	S265	G266	V268	A270	A273	T274		
	sav19.1	A16	H17	R19	G20	L21	T22	G23	S24	G25
20	V26	K27	V28	S87	A88	E89	H120	V121	A232	L233
	V234	K235	Q236	K237	N238	P239	T274			
	sav19.2	A1	Q2	S3	V4	P5	D41	H64	H67	G70
25	T71	A74	L75	N77	S78	I79	G80	V81	L82	G83
	G202	V203	N204	V205	Q206	S207	T208	Y209	Y214	A215
	S216	L217	N218	G219	M222					

30 For PD498, the following amino acid residues belong to the epitope area that correspond to each epitope sequence indicated in Table 3:

161

	pd1.1	D105	A108	S109	G110	I111	R112	Y113	A114	A115	D116
		Q117	N131	S132	T133	T134	L135	K136	S137	A138	V139
		D140	Y141	A142	W143	N144	K145	G146	A147		
5	pd1.2	C128	E129	A153	G154	N155	D156	N157	V158	S160	R161
		T162	F163	Q167	S170	G178	A179	I180	D181	D184	R185
		K186	A187	S188	F189	S190	N191	Y192	G193	T194	W195
		V196	T220	T262	N263						
10	pd1.3	F50	L104	D105	S106	I107	A108	S109	G110	I111	R112
		Y113	A114	A115	D116	Q117	T133	T134	L135	K136	S137
		A138	V139	D140	Y141	A142	W143	N144	K145	G146	A147
15	pd1.4	T28	*28aV	A29	V30	D32	S33	G34	V35	Y37	
		*44aaV		I45	K46	G47	Y48	D49	F50	I51	R53
		D54	N55	N56	P57	M58	D60	L61	K89	I90	L91
		A92	V93	R94	V95	L96	D97	A98	Y113	A114	Q117
20		A119									
25	pd1.5	D32	S33	G34	K46	G47	Y48	D49	F50	I51	D52
		R53	D54	N55	P57	M58	L61	L91	A92	V93	R94
		V95	L96	D97	A98	L104	D105	S106	I107	A108	S109
		G110	I111	R112	Y113	A114	A115	D116	Q117	G118	A119
		T133	T134	L135	K136	S137	A138	V139	D140	Y141	A142
30	pd2.1	V19	T21	I111	R112	Y113	A114	A115	D116	Q117	G118
		A119	L122	D140	Y141	A142	W143	N144	K145	G146	A147
		V148	L233	L234	A235	S236	Q237	G238	K239	N240	N243
		V244	Q245	I246	R247	Q248	A249	A273	V274	R275	Y276

5	pd2.2	S24	S25	T26	Q27	T28	*28aV	L42	A43	R44	*44aK
		*44aaV		I45	D75	N77	D87	T88	K89	I90	L91
		G118	A119	K120	V121	L122	G146	A147	V148	A232	A235
		S236									
10	pd2.3	R22	G23	S24	S25	T26	Q27	T28	*28aV	D87	T88
		K89	I111	A115	G118	A119	K120	V121	L122	S137	A138
		V139	D140	Y141	A142	W143	N144	K145	G146	A147	V148
		V149	V150	I175	A231	A232	A235	S236	N243	I246	R247
15	pd2.4	W-6	S12	T13	P14	A15	A16	V19	T21	R22	G23
		S24	Q27	L230	A231	L233	L234	A235	S236	Q237	G238
		K239	N240	N243	Q245	I246	S270	N271	K272	A273	V274
		R275	Y276								
20	pd3.1	L31	K46	G47	Y48	F50	L91	V93	S103	L104	D105
		S106	I107	A108	S109	G110	I111	R112	Y113	A114	A115
		D116	Q117	G118	L122	L124	C130	S132	T133	T134	L135
		K136	S137	A138	V139	D140	Y141	A142	Q167	P168	Y171
25		P172									
30	pd3.2	V19	T21	R22	G23	S24	Q27	K120	V121	V148	L230
		A231	A232	L233	L234	A235	S236	Q237	G238	K239	N240
		N243	Q245	I246	R247	Q248	A249	I250	Q252	T253	K272
		A273	V274	R275	Y276						
	pd4.1	W-6	S12	T13	P14	A15	A16	W17	D18	V19	T21
		R22	G23	S24	M84	A85	P86	D87	T88	A142	W143

164

	pd5.0	F50	S103	L104	D105	S106	I107	A108	S109	G110	I111
		R112	Y113	A114	A115	D116	Q117	T133	T134	L135	K136
		S137	A138	V139	D140	Y141	A142				
5	pd6.1	Y4	Y6	G7	G63	H64	H67	V68	T71	N155	A179
		F189	P201	G202	V203	N204	I205	A206	S207	V209	G213
		Y214	S215	Y216	M217	S218	G219	T220	S221	M222	A223
		S224	P225	H226							
10	pd6.2	W-6	T13	A16	W17	V19	T21	R22	G23	S24	S25
		Q27	M84	A85	P86	D87	T88	G229	L230	A231	A232
		L233	L234	A235	S236	Q237	G238	S270	V274		
15	pd7.0	R22	G23	S24	S25	Q27	T28	*28aV	A29	V30	V35
		D36	Y37	N38	H39	P40	D41	L42	A43	R44	*44aK
		*44aaV		T66	A69	G70	V72	A73	A74	D75	N77
		A85	P86	D87	T88	K89	I90	L91	A119	V121	L122
		N123	T208	A228	A231						
20											
	pd8.0	W-6	T13	A16	W17	T21	R22	G23	Q27	*44aK	A73
		A74	*75aT	G83	M84	A85	P86	D87	T88	K120	V121
		I175	A176	V177	G178	V196	D197	V198	T199	A200	V227
25		G229	L230	A231	A232	L233	L234	A235	S236	Q237	G238
		K239	N240	N243	Q245	I246	Q248	A249	I250	Q252	T253
		A254	F264	Y265	G266	I268					
30	pd9.0	W-6	Y6	G7	P8	Q9	N10	T11	S12	T13	P14
		A15	A16	W17	D18	V19	T21	M84	V139	W143	V148
		V149	A151	P168	A169	Y171	P172	N173	A174	I175	A176
		D181	S182	N183	D184	D197	P201	L230	L233	L234	K239
		N240	N243	V244	Q245	I246	R247	Q248	A249	I250	E251

165

Q252 T253 A254 K267 I268 N269 S270 N271 K272 A273
V274 R275 Y276

pd10.0 L124 L126 G127 C128 E129 C130 N131 L135 V139
5 A151 A152 A153 G154 N155 D156 N157 V158 S160 R161
T162 F163 Q167 P168 A169 S170 Y171 A174 I175 A176
N191 Y192 G193 T194 W195 V196 T262 N263 F264
*264aK

10

pd11.0 W-6 S-5 Y2 Y4 Q5 Y6 G7 P8 Q9
N10 T11 S12 T13 P14 W17 D18 V19 T21 A82
M84 I180 D181 S182 N183 D184 P201 G202 V203 N204
I205 H226 L233 S270 N271 V274 R275

15

pd12.0 G127 C128 E129 V139 V148 V149 V150 A151 A152
A153 G154 N155 D156 V158 R161 T162 F163 Q167 P168
A169 S170 Y171 P172 N173 A174 I175 A176 V177 G178
20 N191 Y192 G193 T194 W195 V196 D197 V198 T199 A200
V227 R247 I250 E251 A254 N263 F264 *264aK Y265
G266 I268

pd13.1 W-6 S-5 P-4 D-2 P-1 Y1 Y2 S3 *3aA
25 Y4 Q5 P8 Q9 S12 T13 P14 A15 A16 W17
D18 V19 T21 R22 G80 V81 A82 N271 V274 R275

30 pd13.2 W-6 S-5 P-4 N-3 D-2 P-1 Y1 Y2 S3
*3aA Y4 Q5 P8 Q9 P14 W17 D41 G70 A74
D75 *75aT N76 N77 G78 I79 G80 V81 A82 G83
A206 S207 T208 Y214

166

5	pd14.0	T28	V35	D36	Y37	N38	H39	P40	D41	L42
	A43	R44	*44aK	*44aaV		I45	K46	G47	Y48	D49
	F50	R53	D54	N55	N56	P57	M58	T66	A69	G70
	A73	A74	D75	K89	I90	L91	A92	V93	R94	Y113
	T208									
10	pd15.0	V30	L31	D32	S33	G34	V35	D36	Y37	N38
	H39	L42	A43	*44aaV		K46	Y48	D49	F50	I51
	N56	P57	M58	D60	L61	N62	G63	H64	G65	T66
	A69	I90	A92	V93	R94	V95	L96	D97	A98	G100
	S101	G102	S103	S106	I107	G110	S125	L126	V209	P210
	N211	N212								
20	pd16.0	W-6	S-5	P-4	N-3	Y2	G7	P8	Q9	N10
	T11	S12	T13	P14	A15	A16	W17	D18	V19	T21
	R22	*75aT	N76	A82	G83	M84	A85	P86	L233	N269
	S270	N271								
25	pd17.1	T11	S12	A15	A16	D18	V19	T21	R22	G23
	S24	Q27	L230	A232	L233	L234	A235	S236	Q237	G238
	K239	N240	N243	Q245	I246	Q248	A249	Q252	T253	N269
	S270	N271	K272	A273	V274	R275	Y276			
30	pd17.2	A108	I111	R112	A115	D116	K120	L124	T133	T134
	L135	K136	S137	A138	V139	D140	Y141	A142	W143	N144
	K145	G146	A147	V148	V149	P168	Y171	N173	A174	N243

167

pd18.1	W-6	T13	A16	W17	V19	T21	R22	G23	S24
S25	*44aK	M84	A85	P86	D87	T88	K89	G229	L230
A231	A232	L233	L234	A235	S236	Q237	K239	A249	I250
T253	N269	S270	N271	K272	A273	V274	R275	Y276	
5									
pd18.2	D-2	V30	V35	D36	Y37	N38	H39	P40	D41
L42	A43	R44	*44aK	*44aaV		I45	K46	G47	Y48
P57	T66	A69	G70	A73	A74	D75	*75aT	N76	N77
10	I79	V81	A82	A85	P86	D87	T88	K89	I90
	A92	V93	R94	T208					

For Lipolase, the following amino acid residues belong to the
 15 epitope area that correspond to each epitope sequence indicated
 in Table 4:

lip2.1	Y53	F55	V63	L78	F80	W117	V120	A121	D122
T123	L124	R125	Q126	K127	V128	E129	D130	A131	V132
20	R133	V140	L159	R160	G161	N162	G163	Y164	D165
	G190								

25 lip2.2	V2	L6	F10	A173	P174	R175	A182	L193	Y194
R195	I196	T197	P204	R205	Y213	S214	H215	S216	S217
P218	E219	Y220	W221	I222	I235	V236	K237	I238	E239
I241	D242	A243	G246	N247	N248				

30

lip2.3	V2	L6	F10	A182	L185	T186	L193	Y194	R195
I196	T197	H215	S216	S217	P218	E219	Y220	W221	I222
I235	V236	K237	I238	E239	G240	I241	A243	G246	N247

168

N248

5 lip2.4 V2 L6 F10 L193 Y194 R195 I196 T197 S216
 S217 P218 E219 Y220 W221 I222 I235 V236 K237 I238
 E239 G240 A243 G246 N247 N248

10
 lip3.0 L93 K94 F95 H110 A173 P174 R175 V176 G177
 N178 R179 A182 L185 T186 L193 R195 N200 D201 I202
 P204 R205 L206 P207 P208 R209 E210 F211 G212 Y213
 S214 H215 S216 S217 P218 E219 I238 E239 G240 I241
 15 D242 A243 T244 G245 N248 ?R259? P250 N251 I252
 P253 D254 I255

 lip4.0 R175 V176 G177 N178 R179 A180 F181 A182 E183
 20 F184 L185 T186 R205 P207 P208 R209 E210 F211 G212
 Y213 S214 H215 S216 S217 I241 D242 N248

25 lip5.1 A20 Y21 N25 N26 T50 F51 L52 Y53 S54
 F55 E56 V63 T64 G65 F66 L67 A68 L69 I76
 V77 L78 S79 F80 R81 G82 S83 R84 S85 I86
 E87 N88 W89 K127 V128 A131 H145 S146 L147 G148
 L151 G266

30
 lip5.2 K94 F95 L96 L97 K98 E99 R108 G109 H110
 D111 G112 R175 V176 G177 N178 R179 A180 F181 A182
 E183 F184 R205 P207 P208 R209 E210 F211 G212 Y213

169

S214 H215 S216 I241 D242 N248

5	lip6.0	Q9	F10	N11	F13	A14	S17	V63	F80	R81
		W89	L93	F113	S116	W117	F142	T143	G144	H145
		L147	G148	G149	A150	L151	A152	T153	V154	A155
		A157	V168	F169	S170	Y171	G172	A173	P174	R175
		F181	L185	L193	Y194	R195	I196	T197	D201	V203
10		L206	P207	H215	H258	Y261	F262	I265		
	lip7.0	F13	A14	Q15	Y16	S17	A180	A19	A20	Y21
		C22	G23	N25	N26	I34	C36	A40	C41	F51
15		Y53	S54	F55	E56	V63	T64	G65	F66	L67
		F80	R81	V120	A121	D122	T123	L124	R125	Q126
		V128	L264	I265						
20	lip8.1	L12	F13	A14	Q15	Y16	S17	A18	A19	A20
		I34	V44	A49	T50	F51	L52	F66	L67	A68
		D70	N71	T72	N73	K74	L75	I76	V77	S79
		P136	D137	Y138	R139	V140	V141	T143		
25										
	lip8.2	L12	F13	A14	Q15	Y16	S17	A18	A19	A20
		I34	V44	A49	T50	F51	L52	Y53	S54	F55
		F66	L67	A68	L69	D70	N73	L75	I76	V77
30		S79	T123	L124	R125	Q126	K127	V128	E129	D130
		T143								

170

5	lip9.0	L6	F10	N25	N26	D27	A28	A30	G31	T50
		F51	L52	Y53	S54	F55	E56	G65	F66	L67
		L69	I76	T123	L124	R125	Q126	K127	V128	E129
		A131	V132	R1333	E134	H135	P136	R139	V140	V141
		G156	L159	R160	G161	N162	G163	Y164	D165	I166
		V168	F169	S170	G190	G191	T192	L193	Y194	R195
		Y220								
10	lip10.0	N11	L12	Q15	Y16	I34	T35	C36	C41	P42
		E43	V44	E45	K46	A47	D48	A49	D70	N71
		N73	K74							T72
15	lip11.0	F95	L96	L97	K98	E99	I100	N101	D102	C107
		R108	G109	H110	D111	F113	T114	S115	A150	T153
		A173	P174	R175	V176	G177	N178	R179	F181	V203
		R205	L206	P207	P208	R209	F211	G212	Y213	S214
		G240	I241	D242	A243	T244	N248			H215
20	lip12.0	L96	L97	K98	E99	I100	N101	D102	C104	S105
		G106	C107	R108	G109	H110	T114	S115	V176	G177
		A180	F181	F184						N178
25	lip13.0	N11	L12	F13	A14	Q15	Y16	S17	A182	A19
		A20	Y21	N26	I34	C36	A40	C41	P42	E43
		A49	F55	E56	V63	T64	G65	F66	L67	A68
		N73	L75	I76	V77	L78	S79	F80	R81	G82
		R84	W89	W117	L124	V128	V141	F142	T143	G144
		S146	L147	G148	G149	A150	L151	A152	A155	H145
30	lip13.0	N11	L12	F13	A14	Q15	Y16	S17	A182	A19
		A20	Y21	N26	I34	C36	A40	C41	P42	E43
		A49	F55	E56	V63	T64	G65	F66	L67	A68
		N73	L75	I76	V77	L78	S79	F80	R81	G82
		R84	W89	W117	L124	V128	V141	F142	T143	G144
		S146	L147	G148	G149	A150	L151	A152	A155	H145

171

	lip14.0	Q9	F10	N11	F13	A14	S17	Y21	R81	G82
	S83	R84	S85	I86	E87	N88	W89	I90	G91	N92
	L93	F113	T143	G144	H145	S146	L147	G149	A150	T153
5	V168	F169	S170	Y171	A173	P174	R175	V176	L193	Y194
	R195	I196	T197	D201	V203	P204	L206	P207	H215	H258
	Y261	F262	I265	G266						
	lip15.0	N11	L12	F13	A14	Q15	Y16	S17	A18	A19
10	A20	Y21	C22	G23	K24	N25	N26	D27	A28	I34
	T35	C36	A40	C41	P42	E43	V44	E45	K46	A47
	A49	F51	L52	Y53	S54	F55	E56	T64	G65	F66
	L67	S79	F80	R81	T123	L124	K127	L264	I265	
15	lip16.0	A14	E87	I90	H145	G172	I196	T197	H198	T199
	N200	D201	I202	P204	R205	W221	I222	K223	S224	G225
	T226	G246	N247	N254	I252	P253	D254	I255	P256	A257
	H258	L259	W260	Y261	F262	G263	I265			
20	lip17.0	E1	V2	F7	F10	G177	N178	R179	A180	F181
	A182	E183	F184	L185	T186	L193	R195	H198	T199	G212
	S214	H215	S216	S217	P218	E219	Y220	W221	I222	K223
	S224	G225	T226	V228	P229	V230	T231	R232	N233	D234
25	I235	V236	K237	I238	E239	G240	I241	D242	A243	T244
	G245	G246	I262							
	lip18.0	Q9	F13	Y16	T32	N33	I34	C41	P42	E43
	V44	E45	K46	A47	D48	A49	T50	F51	L52	L67
	A68	L69	D70	N71	T72	N73	L75	I76	V128	V132
30	H135	P136	D137	Y138	R139	V140	V141	F142	Y164	D165
	I166	D167	F169	Y194						

For Amylase, the following amino acid residues belong to the epitope area that correspond to each epitope sequence indicated in Table 5:

5	je1.1	N2	G3	T4	R33	P346	Y349	I352	L353	T354	R355
		P360	V362	D366	Y367	M378	K379	A380	K381	I382	D383
		P384	I385	L386	E387	A388	R389	Q390	N391	F392	A393
		Y394	I450	T451							
10	je1.2	Y57	D58	Y60	D61	F65	N66	Q67	L104	G105	G106
		A107	D108	A109	T110	E111	A135	W136	T137	K138	F139
		D140	F141	P142	G143	R144	G145	N146	T147	Y148	S149
		F151	K152	W153	R154	F158					
15	je2.1	M6	Y8	E10	W11	H12	D26	L30	R33	V325	D326
		N327	H328	D329	S330	Q331	P332	G333	E334	E337	F339
		K345	Y349	V362	F363	Y364	G365	D366	Y367	Y368	G369
		I370	P371	T372	H373	S374	V375	P376	A377	M378	K379
		I382	D383	L386							
20											
	je2.2	L289	L293	V314	P318	T323	F324	V325	D326	F339	K345
		P346	L347	A348	Y349	A350	L351	I352	L353	T354	R355
		F356	Q357	G358	Y359	P360	S361	V362	F363	Y364	G365
		D366	Y367	Y368	G369	P376	A377	M378	K379	I382	I385
25		R389	Q397								
	je2.3	N102	V116	E117	V118	P120	R123	D159	G160	V161	D162
		W163	Q168	F169	Q170	N171	R172	I173	Y174	K175	A182
		W183	D184	V187	D188	N193	Y194	D195	Y196	L197	M198
30		Y199	A200	D201	V202	H236					
	je2.4	T1	N2	T4	M6	Y8	D26	L30	R31	N32	R33
		G34	I35	V325	D326	F339	K345	Y349	L353	V362	F363
		Y364	G365	D366	Y367	Y368	G369	I370	P376	A377	M378

173

K379 I382 D383 P384 I385 L386 E387 A388 R389 Q390
 N391 F392 Y394 H417

je3.1 M6 Q7 Y8 F9 E10 L13 H19 W20 N21 R22
 5 L23 R24 D25 D26 A27 S28 N29 L30 R31 N32
 R33 I385 W39 I40 P41 P42 A43 W44 V52 G53
 Y54 Y75 A87 L88 N91 V93 D98 V100 Y364 Y368

10 je3.2 Y8 F9 W11 H19 W20 W39 I40 P41 P42 A43
 W44 D51 V52 G53 Y54 G55 A56 Y75 D98 V99
 V100 M101 N102 H103 L104 D195 L197 M198 A200 D201
 V202 R230 I231 D232 A233 V234 K235 H236 I237 E262
 H328

15

je3.3 Y8 F9 H19 W20 W39 I40 P41 P42 A43 W44
 K45 G46 T47 V52 G53 Y54 G55 A56 Y57 D58
 L59 Q67 K68 Y75 D98 V100 L104 G105 G106 A107
 D108 A109 T110 E111 A135 W136 T137 K138 F139 D140
 20 F141 P142

je4.1 L23 D25 D26 A27 S28 N29 L30 R31 N32 R33
 G34 I35 T36 I38 A84 I85 H86 A87 L88 K89
 N90 N91 G92 V93 Q94 V95 Q390

25

je4.2 A43 W44 K45 L59 Y60 D61 L62 G63 E64 F65
 V71 R72 T73 K74 Y75 G76 T77 R78 S79 Q80
 L81 E82 S83 Y148 W219 Y220 T223 L224

30

Example 4

5 Having identified 'antibody binding peptide' sequences (e.g. "SDFGHKV") and by consensus analysis also "epitope patterns" (e.g. >DF>>K>), one can identify potential epitope sequences on the 3-dimensional surface of a parent protein (=acceptor protein) in a semi-automated manner using the following method:

10

The anchor amino acid residues are transferred to a three dimensional structure of the protein of interest, by colouring D red, F white and K blue. Any surface area having all three residues within a distance of 18Å, preferably 15Å, more preferably 12Å,
15 is then claimed to be an epitope. The relevant distance can easily be measured using e.g. molecular graphics programs like InsightII from Molecular Simulations Inc.

The residues in question should be surface exposed, meaning that
20 the residue should be more than 20% surface exposed, preferably more than 50% surface exposed, more preferably 70% surface exposed. The percentage "surface accessible area" of an amino acid residue of the parent protein is defined as the Connolly surface (ACC value) measured using the DSSP program to the relevant protein part of the structure, divided by the residue total surface area and multiplied by 100. The DSSP program is disclosed in W. Kabsch and C. Sander, BIOPOLYMERS 22 (1983) pp. 2577-2637. The residue total surface areas of the 20 natural amino acids are tabulated in Thomas E. Creighton, PROTEINS; Structure and Molecular Principles, W.H. Freeman and Company, NY, ISBN: 0-7167-1566-X (1984).
25
30

Substitutions of one or more residue (s) within 18Å, preferably 15Å, more preferably 12Å, around the geometrical center of the

residues involved in the epitope, for a bigger or smaller residues, may destroy the epitope, and make the protein less antigenic.

- 5 Residues involved in epitope is 2, preferably 3 and more preferably 4

Example 5

10

Production, selection, and evaluation of enzyme variants with reduced antigenicity or immunogenicity.

Epitope sequences and hot-spots amino acids were mutated using
15 standard techniques known to the person skilled in the field (e.g. site-directed mutagenesis, error-prone PCR- see for example Sambrook et al. (1989), Molecular Cloning. A Laboratory Manual, Cold Spring Harbour, NY).

- 20 In the examples shown below, variants were made by site-directed mutagenesis. Amino acid exchanges giving new epitopes or duplicating existing epitopes, according to the information collected in the epitope-database (See Example 1), were avoided in the mutagenesis process.

25

Enzyme variants were screened for reduced binding of antibodies raised against the backbone enzyme. Antibody binding was assessed by competitive ELISA as described in the Methods section.

- 30 Variants with reduced antibody binding capacity were further evaluated in the mouse SC animal model (See methods section).

The following variants showed reduced IgE and/or reduced IgG levels in the mouse model:

Parent protein	Mutations	Target epitope sequences	%IgG re-sponse	%IgE re-sponse
Savinase	D181N	Sav11.0; Sav15.0 and Sav18.1. Hot spot amino acid.	50	19
Savinase	R170L; Q206E	Sav9,4; Sav10,4; Sav1.1; and Sav19.2	5	34
Savinase	R170L, S57P	Sav9,4; Sav10,4	45	12
Savinase	R247E	Sav2.3, Sav6.1, Sav18.2 Hot spot amino acid.	75	30
Savinase	R247Q	Sav2.3, Sav6.1, Sav18.2 Hot spot amino acid.	17	20
Savinase	R247H	Sav2.3, Sav6.1, Sav18.2 Hot spot amino acid.	40	27
Savinase	R247K	Sav2.3, Sav6.1, Sav18.2 Hot spot amino acid.	74	34

Production, selection, and evaluation of enzyme variants with reduced antigenicity or immunogenicity.

Hot-spots or epitopes were mutated using techniques known to the expert in the field (e.g. site-directed mutagenesis, error-prone PCR).

In the examples showed below, variants were made by site-directed mutagenesis. Amino acid exchanges giving new epitopes or duplicating existing epitopes according to the information collected in the epitope-database, were avoided in the mutagenesis process.

Enzyme variants were screened for reduced binding of antibodies raised against the backbone enzyme. This antibody binding was assessed by established assays (e.g. competitive ELISA, agglutination assay).

Variants with reduced antibody binding capacity were further evaluated in animal studies.

Mice were immunised subcutaneous weekly, for a period of 20 weeks, with 50 µl 0.9% (wt/vol) NaCl (control group), or 50 µl 0.9% (wt/vol) NaCl containing 10 µg of protein. Blood samples (100 µl) were collected from the eye one week after every second immunization. Serum was obtained by blood clotting, and centrifugation.

Specific IgG1 and IgE levels were determined using the ELISA specific for mouse or rat IgG1 or IgE. Differences between data sets were analysed by using appropriate statistical methods.

A. Site-directed mutagenesis of amino acids defining epitopes,
with an effect on IgG1 and/or IgE responses in mice.

5

Epitope: A172/A169 R170 A194 G193 N261

Pattern: A R > R > A > N

Antibody: IgG1 + IgE

Backbone: Savinase

10

The variant carried mutation R170F.

In a competitive IgE ELISA, this variant was less effective in competing for anti-savinase antibodies, giving a 15% lower end-point inhibition as compared to the savinase backbone.

15

Mouse studies revealed an 80% reduction of the specific IgE levels, as compared to savinase backbone ($p < 0.01$). The IgG1 levels were not significantly affected.

20

Epitope: S216 E219 Y220

Pattern: E Y > M

Antibody: IgG1

25 Backbone: Lipoprime

The variant carried mutation S216W.

In a competitive IgG ELISA, the variant was less effective in competing for Lipolase antibodies, giving a 38% decrease in
30 endpoint inhibition as compared to the enzyme backbone.

Mouse studies revealed a 69% decrease in specific IgG1 levels, compared to the lipolase backbone ($p < 0.05$). The IgE levels were not significantly affected.

B. Site-directed mutagenesis of epitopes, with examples of epitope duplication, and new epitope formation, respectively, predicted by the epitope-database.

10

Epitope: T143 N173 N140 E136 L135

Pattern: S/T N N > E L

Antibody: IgG1

15 Backbone: Savinase

The variant carried mutation E136R.

In a competitive IgG ELISA, the variants was less effective in competing for savinase antibodies, giving a 38% decrease in endpoint inhibition as compared to the savinase backbone.

Mouse studies revealed a dramatic increase in specific IgG1 levels, compared to savinase backbone ($p < 0.01$). The IgE levels were not significantly affected.

25

Mutation E136R establishes an IgG1 epitope of the R Y P R/K pattern, previously identified on PD498. Apparently, this new epitope was more antigenic in mice than the existing epitope. The introduction of a savinase unrelated epitope on the savinase backbone could explain the observed discrepancy between competitive ELISA and animal studies.

In this example, it was found that using information derived exclusively from screening phage libraries with anti-PD498 anti-

bodies (to identify the R Y P R/K epitope pattern of Table 2) one could predict the outcome of a genetic engineering experiment for Savinase in which the E136R mutation created the PD498-epitope on the Savinase surface, leading to increased immunogenicity of this Savinase variant. This demonstrates that the epitope patterns identified may be used to predict the effect on immunogenicity of substitutions in proteins that are different from the parent protein(s) used to identify the epitope pattern.

C. Site-directed mutagenesis of amino acids defining epitope areas, with a differential effect on IgG1 and IgE antibody levels in mice, and an inhibiting effect on IgG binding, respectively.

Epitope: A172/A169 R170 A194 G193 N261

Pattern: A R > R > A > N

Antibody: IgG1 + IgE

10 Backbone: Savinase

Epitope area: P131, S132, A133, L135, E136, V139, A151, A152, S153, G161, S162, I165, S166, Y167, P168, Y171, N173, A174, A176, Q191, Y192, G195, L196, R247, S259, T260, L262, Y263, G264.

15

The variant was different at position Y167 by the mutation Y167I.

In a competitive IgE ELISA, the variant was less effective in competing for anti-savinase antibodies, giving a 8% lower end-point inhibition as compared to the its backbone.

Mouse studies revealed an 75% reduction of the specific IgE levels, as compared to the backbone ($p < 0.01$). In contrast, the IgG1 levels were dramatically increased ($p < 0.01$).

25

Epitope: T143 N173 N140 E136 L135

Pattern: S/T N N > E L

Antibody: IgG1

Backbone: Savinase

30 Epitope area: V10A, I107, A108, L111, E112, G115, S132, A133, T134, Q137, A138, V139, S141, A142, S144, R145, G146, V147, V149, Y167, P168, Y171, A172, A174, M175, N243, R247.

While variant no. 1 was mutated at the epitope position (N140D), variant no. 2 was mutated at N140 (N140D), but also at the epitope area position (A172D).

5 In a competitive IgG ELISA, variant no. 1 was less effective in competing for anti-savinase antibodies, as compared to savinase. This variant revealed a 21% lower endpoint inhibition as compared to the its backbone.

10 Variant no. 2 resulted in an endpoint inhibition that was 60% lower as compared to savinase, and 40% as compared to variant no. 1.

15 Example 7

Conjugation of Savinase variant E136K with activated bis-PEG-1000

4,9 mg of the Savinase variant was incubated in 50 mM Sodium Borate pH 9.5 with 12 mg of N-succinimidyl carbonate activated bis-PEG 1000 in a reaction volume of approximately 2 ml. The reaction was carried out at ambient temperature using magnetic stirring while keeping the pH within the interval 9.0-9.5 by addition of 0.5 M NaOH. The reaction time was 2 hours.

25 The derivatives was purified and reagent excess removed by size exclusion chromatography on a Superdex-75 column (Pharmacia) equilibrated in 50 mM Sodium Borate, 5mM Succinic Acid, 150 mM NaCl, 1 mM CaCl₂ pH 6.0.

The conjugate was stored at -20°C, in the above described 30 buffer.

Compared to the parent enzyme variant, the protease activity of the conjugate was retained (97% using Dimethyl-casein as substrate at pH 9).

Example 8

Competitive ELISA was performed according to established procedures. In short, a 96 well ELISA plate was coated with the parent protein. After proper blocking and washing, the coated antigen was incubated with rabbit anti-enzyme polyclonal antiserum in the presence of various amounts of modified protein (the competitor).

10

The amounts of residual rabbit antiserum was detected by pig anti-rabbit immunoglobulin, horseradish peroxidase labelled.

Epitope: T143 N173 N140 E136 L135
15 Pattern: S/T N N > E L
Antibody: IgG1
Backbone: Savinase

Mutation: E136K
20 Modification: bis-NHS-PEG1000

The data show that the derivative (60% endpoint inhibition) has reduced capacity to bind enzyme specific immunoglobulines, as compared to the parent protein (100% endpoint inhibition).

Example 9

30 For this example the epitope sequences were determined in four environmental allergens (Bet v1; Der f2; Der p2 and Phl p2), based on their structures (1btv.pdb; 1ahm.pdb; a19v.pdb; and 1whp.pdb, respectively), sequences (SEQ ID NO: 6, 7, 8 and 9, respectively) and computer modelling of the epitope patterns

that had been assembled in our database (shown in Table 8). The allergens arise from common sources of allergy: Birch (Bet v1 from *Betula pendula*), House dust mites (Der f2 from *Dermatophagoides farinae* and Der p2 from *Dermatophagoides pteronyssinus*), and Timothy grass (Phl p2 from *Phleum pratense*).

The protein surface is scanned for epitope patterns matching the given "consensus" sequence of about 6-12 residues. First, residues on the protein surface that match the first residue of the consensus sequence are identified. Within a specified distance from each of these, residues on the protein surface that match the next residue of the consensus sequence are identified. This procedure is repeated for the remaining residues of the consensus sequence. The method is further described under the paragraph "Methods" above and the computer program can be found in the Appendixes.

The critical parameters used in this screening included:

- i) a maximal distance between the alpha-carbon atoms of subsequent amino acids,
- ii) a minimal accessibility of the amino acid of 20\AA^2 ,
- iii) the largest maximal distance between the most distinct amino acids should be less than 25\AA
- iv) the 5 best epitopes were taken,
- v) the minimal homology with the epitope pattern of interest was 80%

In this way a number of potential epitopes are identified. The epitopes are sorted according to total surface accessible area, and certain entries removed:

- 1) Epitopes that contain the same protein surface residue more than once. These are artefacts generated by the described algorithm.

185

- 2) Epitopes which are "too big", i.e. where a distance between any two residues in the epitope exceeds a given threshold.

The epitope sequences found by this second generation mapping
5 procedure were:

The epitope sequences found were:

10 Bet v1:

Epi#02

A146, K32, Q36, F30, T142, R145, V12

A34, K32, Q36, F30, T142, R145, V12

15

Epi#03

L62, K65, ---, I56, Y66

L24, K20, H76, I23, Y81

L24, K20, H76, I104, Y81

20

Epi#04

K134, S136, Q132, K129, A130, A135

K134, S136, Q132, K129, V128, G1

25 Epi#05

G140, A146, R145, T10, G111, A106, T107, V12

G26, A146, R145, T10, G110, A106, T107, V12

G140, A146, R145, T10, G110, S11, S149, L152

G110, A106, S11, T9, G140, R145, T10, V12

30 G140, A146, R145, T10, G111, S11, S149, V12

Epi#06

G110, P108, D109, T107, A106, P14

G111, P108, D109, T107, A106, P14

186

A34, N28, D27, S40, K32, P35
G26, N28, D27, S39, K32, P35
A106, N78, D75, T77, A16, P14
G26, N28, D27, S39, Q36, P35

5

Epi#07
G46, T52, D69, S99, R70, V71, P50, D72
G49, T52, D69, S99, R70, V71, P50, D72
G48, T52, D69, S99, R70, V71, P50, D72

10

Epi#08
K123, E127, G1, V2, H121, F3
K65, E60, F64, V67, F58
K65, E60, F58, V67, F64

15 K129, E127, G1, V2, H121, F3

Epi#09

S149, L152, D156, N159, R17, L24, D75, K103, N78, A106, V12
L152, S149, D156, N159, R17, L24, D75, H76, N78, A106, V12

20 L152, D156, N159, R17, L24, D75, K80, N78, A106, V12

Epi#10

D109, A106, N78, T77, F79, R17, K20
E141, T10, R145, T142, F30, G26, K32

25 E8, T10, R145, T142, F30, G26, K32

Epi#11

F30, K32, I38, Q36, V33, E148
F22, F30, I38, Q36, V33, E148

30 F30, L143, I38, Q36, V33, E148

Epi#12

Y5, E6
Y83, E73

Y120, E127

Y5, E8

Y66, E87

Y81, E73

5

Epi#13

H76, A16, P14, T107, A106, P108, G110, G111

A16, R17, P14, T107, A106, P108, G110, G111

A157, R17, P14, T107, A106, P108, G111, G110

10

Epi#15

K65, P90, D93, I91, K97, G92

K32, P31, D27, I56, K65, G61

15 Epi#17

A153, S149, R145, S11

A106, S11, R145, S149

Epi#18

20 R145, S149, L152, A153, Y150, L151, H154, S155

R145, S149, L152, A153, S155, L151, A157, N159

Epi#22

D125, D93, P90, K65

25 D93, P90, P63, E60

Epi#23

K55, N43, E42, S57, L62, P63

K68, N43, E42, S40, F30, P35

30 K54, N43, E42, S57, F64, P63

K55, N43, E42, S40, F58, P35

Epi#24

E96, K97, E87, P90, F64, E60, K65

188

E127, K123, E96, P90, F64, P63, K65
E42, K68, E87, P90, F64, E60, K65
E42, K55, E87, P90, F64, E60, K65
D93, G92, E87, P90, F64, E60, K65
5 D125, K123, E96, P90, F64, P63, K65

Epi#25

R70, K55, I44, E45, E42
R70, K54, I44, E45, N47
10 R70, K68, I53, E45, N47

Epi#27

D93, E127, D125, K123

15 Epi#28

A146, Q36, F58, E60, L62, F64, P63, K65
I38, Q36, F58, E60, L62, F64, P63, K65
A34, Q36, F58, E60, L62, F64, P63, K65
L143, Q36, F58, E60, L62, F64, P63, K65
20 V33, Q36, F58, E60, L62, F64, G61, K65

Epi#29

G61, K65, L62, F58, E60
I56, K65, L62, F64, E60
25 G89, K65, L62, F64, E60
V67, K65, L62, F64, E60

Epi#30

G1, N4, S99, H121, K97, I91, P90
30 I113, I13, S149, H154, S155, L152, L151
I13, L152, A153, H154, S155, L151, V33
G110, I13, S149, H154, S155, L152, L151
G1, N4, S99, H121, K97, I98, V2
G1, N4, S99, H121, K97, I91, V85

Epi#33

K32, F30, P35, S39, S57, K65
Q36, F30, P35, S39, S40, K32
5 K32, F30, P35, S40, S57, K65
K65, F58, P35, S39, A34, R145

Epi#34

V105, P14, T107, V12, R145, Y150, S155
10 I113, P14, T107, V12, R145, Y150, S155

Epi#37

P50, V74, L24, R17, N159
P50, V74, L24, K20, N159
15 P14, R17, L24, K20, N159

Epi#38

L143, G140, E141, R145, V33, N28, P31, S39
L143, G140, E141, R145, V33, N28, P31, S40
20 L143, G140, E141, R145, V33, N28, P31, S57

Epi#39

A130, E127, H126, T94, P90, G89, L62
A130, E127, H121, T94, P90, G89, L62
25

Epi#40

A157, L152, A153, Y150, K32, S39
A153, L152, A157, Y150, K32, S40
R17, L151, A153, Y150, K32, S40
30 R145, L143, A34, Y150, A153, S155
R145, L143, G140, T9, K115, T10

Epi#41

P63, Y66, L62, S57

Epi#44

I23, R17, D156, Y150, S149, V12, T10
L24, R17, D156, Y150, S149, V12, P14
5 L24, R17, D156, Y158, A16, A106, P108
I13, R17, D156, Y150, S149, V12, T10
L151, R17, D156, Y150, S149, V12, T10
L24, R17, D156, Y150, S149, V12, T107

10 Epi#45

K32, P35, F30, Y150, R145, M139, G140
K32, P35, F30, Y150, R145, M139, L143
K32, P31, F30, Y150, R145, M139, G140

15 Epi#47

L152, S149, R145, L143, A34, F30, N28, P31, P35
A153, S149, R145, A146, A34, F30, N28, P31, P35

Epi#48

20 E60, K65, P90, P63, G61
E60, K65, P63, P90, G92

Epi#51

T94, H126, E127, D125, G124, K123, H121
25 D125, H126, E127, T94, K123, T122, H121

Der f2:

30 Epi#02

A98, K100, S101, P99, R128, R31
A98, K100, R128, P99, R31, V94
T91, N93, P95, P34, R31, R128
L61, N93, P95, P34, R31, R128

Epi#03

L40, K15, A39, I13, Y86

L40, K14, A39, I88, Y90

5

Epi#05

G32, A98, R31, P34, G20, T36, T91, Y90

G32, A98, R31, P34, G20, T36, T91, V94

G32, A98, R31, P34, G20, T36, T91, L37

10 G32, A98, R31, P34, G20, T36, T91, V18

Epi#06

A98, P99, D129, R31, K96, P95

G32, P99, D129, R128, R31, P95

15 A98, P99, D129, R31, K33, P95

A98, P99, D129, R31, K96, P34

A98, P99, D129, R128, K126, P26

Epi#07

20 T107, S57, D59, S101, R128, A98, P99, D129

T107, S57, D59, S101, R31, A98, P99, D129

Epi#08

K15, D87, V76, H74, F75

25 K14, D87, V76, H74, F75

K77, D87, V76, H74, F75

Epi#09

L61, D64, I68, H74, F75, T70, N71

30 N114, N46, D113, K48, N71, T70, T49

G83, N46, D113, K48, N71, T70, T49

Epi#10

L40, I13, D42, N44, V81, K48, N46, N114, G115

192

L40, I13, D42, N44, V81, K82, N46, N114, G115
L37, D19, G20, V18, V3, D4, K6, A120, T107, V105

Epi#11

5 F75, K51, I111, Q45, V116, D113
F75, K51, I111, Q45, V81, D113

Epi#12

Y90, E38

10

Epi#13

H30, R31, P95, A98, P99, S101, G60, L61

Epi#15

15 K96, P99, D129, I28, R128, A98
K96, P99, D129, I127, R128, A98
K96, P99, D129, I29, R128, A98
K55, P66, D64, I68, T70, G67

20 Epi#18

R31, R128, I28, G125, T123, H124, V105
R31, R128, I127, G125, T123, H124, V105

Epi#22

25 D1, M17, D4, V3, K6
D1, M17, D19, P34, K96
D1, M17, D4, V5, K6

Epi#23

30 K14, N11, E12, N44, Q85, P79
K14, N11, E12, N10, Q45, P79
K14, N11, E12, N44, Q84, P79
K14, N11, E12, L40, Q85, P79

Epi#24

D129, K100, E102, P99, R128, R31, K96
E62, G60, E102, P99, R128, R31, K96
D129, K126, E102, P99, R128, R31, K33
5 D129, K126, E102, P99, R31, P95, K96

Epi#25

R31, K96, I97, D59, E62
R128, R31, I97, D59, E102
10 R128, K126, I127, E102, N103

Epi#27

D64, E62, D59, K100
D59, E62, D64, K55
15 D87, E38, D19, K33
D19, E38, D87, K15
D19, E38, D87, K14
D19, E38, D87, K77

20 Epi#28

V16, D87, Q85, K14, E12, K15, Q2, D1
I13, D87, Q85, K14, E12, K15, Q2, D1
V3, D1, Q2, K15, E12, K14, Q85, D87
L40, D87, Q85, K14, E12, K15, Q2, D1
25 I88, D87, Q85, K14, E12, K15, Q2, D1
V76, D87, Q85, K14, E12, K15, Q2, D1
V18, D1, Q2, K15, E12, K14, Q85, D87

Epi#29

30 G32, N93, L61, E62
V94, N93, L61, E62

Epi#30

G60, I97, A98, H30, K96, P34, P95

194

I68, N71, H74, K77, P79, V81
G32, I97, A98, H30, K96, P95, P34

Epi#34

5 V105, P26, S24, G125, R128, S101, P99
W92, P34, T91, V94, R31, S101, P99
I28, P26, T123, G125, R128, S101, P99

Epi#37

10 A120, V16, L40, K14, N11
A39, V16, L40, K14, N11
Y90, A39, L40, K14, N11
Y86, A39, L40, K14, N11

15 Epi#39

A120, E38, T91, P34, G20, L37
A39, E38, T91, P34, G20, L37

Epi#40

20 G20, L37, A120, T123, K6, S24
A39, L37, A120, T123, K6, S24
G20, L37, A120, T107, K6, T123

Epi#41

25 P34, L37, V106, S57

Epi#42

P26, S24, G125, R128, R31
P99, S101, G125, R128, R31

30

Epi#44

V16, Q2, D19, P34, W92, Y90, A39, V18, T91
V16, Q2, D19, P34, W92, Y90, A39, V5, T123
V3, Q2, D19, P34, W92, Y90, A39, V18, T91

Epi#45

K77, H74, F75, N71, D69, G67

K77, H74, F75, N71, D69, V76

5 K77, H74, F75, N71, D69, V65

Epi#46

A98, R128, R31, P95, N93, G32

A98, R128, R31, P34, G20, Q2

10

Epi#48

Q2, D19, P34, P95, G32

H30, K96, P95, P34, G20

15 Epi#49

D87, D42, L40, Q85, Q84, C78, T47, Q45, K48

D87, D42, L40, Q85, Q84, C78, T47, Q45, K82

Epi#50

20 D19, W92, P34, T91

D19, W92, P34, P95

D19, W92, T91, T36

Epi#51

25 D129, H30, K33, R31, R128, K126, H124

R31, H30, D129, R128, K100, K126, H124

T123, H124, K126, R128, R31, K33, H30

30 Der p2:

Epi#03

L17, K89, A39, I13, Y86

L17, K89, A72, I88, Y90

L17, K89, A72, I52, Y90

Epi#04

K15, S1, Q2, K14, V16, L17

5 K15, S1, Q2, K14, A39, L17

K15, S1, Q2, K14, V40, I13

Epi#05

G60, A56, L61, P99, G32, R31, H30, I97

10 G60, A56, L61, P99, G32, R31, H30, I28

Epi#06

G60, A56, D64, S57, K55, P66

G83, N46, D114, T49, K48, P79

15 G60, N103, D59, S101, R31, P95

Epi#08

K55, D64, S57, V106, F35

K55, E62, S57, V106, F35

20

Epi#09

L61, G60, E102, R128, I28, K126, N103, T123, V105

L61, G60, E102, R128, I127, K100, N103, T123, V105

L61, G60, E102, R128, I127, H124, N103, T123, V105

25

Epi#10

SAS: 435, Size 24.47: D69, T91, N93, F35, G32, R31

SAS: 422, Size 20.74: E38, T91, N93, F35, G32, K96

30 Epi#11

K14, I13, Q85, V81, E42

K15, I13, Q85, V81, E42

K14, I13, Q85, V40, D87

197

Epi#12

Y86, E42

Y90, E53

Y90, E38

5

Epi#13

H30, A125, P26, T123, A122, P19, L37, P34, W92

H30, A125, P26, T123, A122, H124, S24, G23, G20

H30, A125, P26, T123, A122, P19, L17, G20, F35

10

Epi#15

K55, P66, D69, I68, K89, A72

K55, P66, D69, I68, K89, A39

K55, P66, D64, I54, K109, G115

15 K55, P66, D64, I54, K109, A9

Epi#18

R31, I29, A125, S101, E102, N103

R31, I29, A125, S101, E102, V104

20 R31, I29, A125, T123, A122, V105

Epi#22

D69, P66, D64, V65, K55

D64, P66, D69, T91, K89

25 D59, L61, D64, P66, W92

D59, L61, D64, V65, E62

D69, P66, D64, V65, E53

Epi#24

30 D64, K55, E62, P99, R31, P34, K96

E53, K55, E62, P99, R31, P95, K96

D64, K55, E62, P99, R31, A98, K96

Epi#25

198

R31, H30, I28, E102, N103

R128, K126, I127, E102, N103

R128, K126, I28, E102, V105

5 Epi#27

D64, E53, D69, K89

D69, E53, D64, K55

D59, E62, D64, K55

10 Epi#28

V40, D87, Q85, E42, Q84, G83, K82

G20, H22, Q2, L17, E38, L37, Q36, P34, K33

G20, H22, Q2, L17, E38, L37, F35, P34, K33

15 Epi#29

I97, K100, L61, E62

G60, N103, L61, E62

I127, N103, L61, E62

20 Epi#30

G60, N103, S101, H30, K96, I97, P95

G60, N103, A125, H30, K96, I97, P95

I28, I127, A125, H30, K96, I97, P95

25 Epi#33

Q36, F35, V106, S57, A56, K55

K33, F35, V106, S57, A56, K55

Epi#34

30 I28, P26, S24, G23, G20, T123, S57

I28, P26, S24, V3, G20, T123, T107

W92, P34, T91, V18, G20, T123, P26

Epi#37

199

P66, V63, L61, K100, N103

P95, A98, L61, K100, N103

P19, V18, L17, K89, D87

P19, V3, L17, K89, D87

5 T123, V104, L61, K100, N103

Epi#38

L61, G60, E102, A125, V105, N103, P99, S57

L61, G60, E62, A56, V105, N103, P99, S57

10

Epi#39

A125, E102, H124, T123, P26, G20, L17

Epi#40

15 G60, L61, A56, T107, K6, T123

A39, L17, G20, T123, P26, S24

G60, L61, A56, T107, K55, S57

G60, L61, A56, T123, K126, S101

20 Epi#41

P19, L17, V3, S1

P19, L17, V5, S24

Epi#44

25 V65, D64, P66, W92, Y90, A39, V18, P19

L61, D64, P66, W92, Y90, A39, V18, T91

Epi#45

R31, P34, F35, N93, V94

30 K96, P34, F35, N93, G32

Epi#47

I127, S101, R31, I97, A98, L61, N103, P99, P95

I28, S101, R31, I97, A98, L61, N103, P99, S57

Epi#48

H30, K96, P95, P99, G60
H30, K96, P34, P19, G20
5 H30, K96, P34, P19, V18
H30, K96, P34, P95, V94
H30, K96, P34, P19, V3
E38, K89, P70, P66, V65
H30, K96, P95, P34, G32
10 Q36, K89, P70, P66, V65

Epi#50

D69, Y90, W92, P66, P70
D69, Y90, W92, P34, P95
15 D69, Y90, W92, T91, P34
D69, Y90, W92, V94, P95
D69, Y90, W92, L37, P19

Epi#51

20 K126, H124, E102, R128, I28, R31, H30
T123, H124, K126, R128, I28, R31, H30
D4, H124, K126, R128, I28, R31, H30

25 Ph1 p2:

Epi#02

T87, K85, Q61, S38, R34, R67
T87, K85, Q61, P63, R34, V42

30

Epi#03

K10, A90, I88, Y86
K10, A18, I88, Y86

201

Epi#04

R34, S38, Q61, K85, T87, I88

R34, S38, Q61, K85, T87, A90

5 Epi#05

G47, A18, S12, T87, G89, T91, T5, V1

G73, A29, L69, T27, G50, T53, T45, V42

G11, A18, L20, T91, G89, A90, T87, I88

10 Epi#06

A93, P94, D79, R34, Q61, P59

A93, P94, D79, R34, Q61, P83

A93, P94, D80, R34, Q61, P59

A93, P94, D79, R34, Q61, P63

15

Epi#08

K10, E9, G11, A18, H16, F54

K46, E48, G47, A18, H16, F54

K10, E9, S12, A18, H16, F54

20

Epi#09

L69, T27, G73, N76, R67, V77, D79, R34, A43, T45, V42

L69, T27, A29, E30, R67, V77, D80, R34, A43, T45, V42

25 Epi#10

D55, A18, N13, S12, F54, G47, K46

T45, A18, N13, S56, F54, G47, K46

Epi#09

30 L60, S56, E57, D55, K15, N13, S12, G11

L60, S56, E57, D55, H16, F54, T45, T53

L60, S56, E57, D55, H16, F54, T45, G47

Epi#12

Y86, E84

Y23, E24

Epi#18

5 N76, R67, F78, V81, A93, Y92, T91, T5, P2, V1

Epi#19

D39, W41, S38, Q61, R34, G37

E40, W41, S38, Q61, R34, A43

10

Epi#22

D79, P94, D80, P83, K85

D79, P94, D80, P63, K85

15 Epi#23

K10, N13, E14, L60, Q61, P59

K10, N13, E14, L60, Q61, P83

K10, N13, E14, L60, Q61, P63

20 Epi#24

E58, K15, E57, P59, S56, E14, Q61

D55, K15, E57, P59, S56, E58, Q61

Epi#25

25 R34, R67, W41, D39, E40

Epi#26

S38, E40, W41, V42, E32, E30

S38, E40, W41, V42, A43, E32

30

Epi#27

E14, E57, E58, K15

D55, E14, E84, K85

Epi#28

G37, H36, Q61, K85, E84, L60, F54, A43, K46
G37, H36, Q61, K85, E84, L60, F54, S12, D55
G37, H36, Q61, K85, E84, L60, F54, S56, D55
5 G37, H36, Q61, K85, E84, L60, F54, A43, R67
G37, H36, Q61, K15, E57, L60, F54, A43, K46
G37, H36, Q61, K85, E84, L60, F54, S12, K15
G37, H36, Q61, K85, E84, L60, F54, S56, K15
G37, H36, Q61, K85, E84, L60, F54, A43, R34
10 G37, H36, Q61, K85, E84, L60, F54, A18, D55

Epi#29

G73, K72, L69, R67, E30
I88, N13, L60, F54, E57
15 G25, K72, L69, R67, E32
V77, K75, L69, R67, E30
G37, H36, L60, F54, E57
G37, Q61, L60, F54, E57

20 Epi#30

I88, N13, S12, H16, K15, P59, L60
I88, N13, S56, H16, K15, L60, P59
I88, N13, A18, H16, K15, P59, L60

25 Epi#33

K46, F54, V42, S56, K15
H16, F54, V42, S56, K15

Epi#34

30 V1, P2, T5, V4, P94, Y92, T87
V1, P2, T5, L20, G89, T91, T87
V81, P94, T5, V1, P2, Y92, T91

Epi#37

204

T27, A29, L69, K72, D26
A43, R67, L69, K75, N76

Epi#38

5 L20, G89, E9, A18, N13, P59, S56

Epi#40

G49, L20, G89, Y86, K85, T87
G49, L20, G89, T87, K10, S12
10 G49, L20, G89, T87, K10, T7

Epi#44

V77, R67, D79, P94, Y92, A93, V1, P2
L69, R67, D79, P94, Y92, A93, V1, T5
15

Epi#45

D79, P94, F78, N76, M74, L69
D80, P94, F78, R67, D79, V77
K3, P94, F78, N76, M74, G73
20

Epi#46

A43, R67, R34, P63, H36, Q61
V77, R67, R34, P63, H36, G37
L69, R67, R34, P63, G37, Q61
25

Epi#47

G37, E35, E40, A43, R34, L60, N13, P59, S56
V77, E32, E40, A43, R34, L60, N13, P59, S56
S38, G37, E40, A43, R34, L60, N13, P59, S56
30

Epi#48

E24, K3, P94, P2, V1
E84, D80, P94, P2, V1

205

Epi#50

D39, W41, A43, T45

D39, W41, V42, T45

s Epi#51

D79, H36, E84, T87, K10, G11, H16

D39, H36, Q61, K85, P63, R34, W41

D79, H36, E40, D39, G37, R34, W41

Q61, H36, E84, T87, K10, G11, H16

Table 8: Each row indicates an epitope pattern. At each position (from 1 up to a maximum of 12) the cells indicate which amino acids (single letter coding) that are at that position. The last column indicates the patterns that were identified using IgE antibody binding.

Position	1	2	3	4	5	6	7	8	9	10	11	12
Epitope Pattern Number												
1	TS	RQ	YS	NHC	KR	KR	P	HNP	L			IgE
2	RV	R	Y	PST	FR	ALPQS	RKN	ALT				IgE
3	Y	I	AH	K	L							
4	AGIL	ANRTV	KRY	Q	S	Y	KR					
5	GILVY	STH	ASTR	G	PT	RNAFLS	A	G				IgE
6	P	KRQSA	STRC	D	PAN	GA						IgE
7	D	P	AV	R	S	D	S	T	G			IgE
8	F	HI	VA	FSG	DE	KA						IgE
9	NRGLTV	STAN	ANF	RKH	D	AILV	R	ENRSV	AGI	DGNT	LIS	IgE
10	KR	RG	F	C	AST	RN	NTA	DECT				IgE
11	DE	V	Q	I	FLK	F						IgE
12	E	Y										
13	FWYGL	PG	ALS	PH	A	T	P	LRWA	SAH			IgE
14	GV	Q	ILV	I	Y	GNR	DN	TEH				IgE
15	AG	RKQT	I	D	P	RKN						IgE
16	DN	A	DA	SDN	QRSW	GMR	Y	P	RQL			IgE
17	S	R	S	A								
18	VLSFN	AENPT	T	L	ST	Y	GAL	LIV	CSF	R	FRN	SD
19	AGLKM	R	Q	QSC	NTW	DEI						IgE
20	D	G	D	KN	L	LF	P	K	V	A		IgE
21	P	S	I	I	LR	CI						IgE
22	EDKW	ACLPTWY	D	ASLPM	D							IgE
23	AP	LQF	SYLN	E	N	RK						IgE
24	KQ	AELFPR	TSFR	P	EA	GK	DE					IgE
25	ENV	DE	IW	RKH	R							IgE

Table 8 - continued.

Position	1	2	3	4	5	6	7	8	9	10	11	12
Epitope Pattern												
26	DE	AGE	PHV	W	E-	S	W					
27	K	DE	E	DE								IgE
28	DKR	APSG-	QF-	CFIKLW-	E	FIKLW-	Q	DH-	AGILV			IgE
29	E	RF-	L	KRQHNGP	GILV							IgE
30	LVP	LIP	IKLPQS-	H	AS-	LIMN	GI					IgE
31	D	FI-	MV-	FW	R	N	QR	L				
32	V	f-	DE	A	A	F						
33	KR	SA-	S	VP	YF	KQH						
34	STP	STY	GPR-	GLV	STM	WP	YW					IgE
35	I	M	S	A-	L	AG						
36	AW	A	PV-	K-	Q-	ST	Y-	G-	V-	A	A	TP
37	NYD	KR	L	ARV	TYAP	AR-	E	G	L			IgE
38	S	P	N	LR-	RV-	E	A					
39	L	G	P	RT-	HL-	E						
40	ST-	APK-	YT	AG	L-	AGR						IgE
41	St	V-	L	YH-	P-							
42	RQ	R	P-	H-	NQG	S	P	L				
43	T-	RI	ML	S	HQ	GL	YA	WC	I			
44	PT	AGV	SA	Y	W-	P-	D-	RQ-	ILVS			IgE
45	LVG	MD-	RN	Y-	F	PH	KRD					IgE
46	AGQ	HNQGC	P	R	R	AVLCY	RE	AGSYLE	LIAGVS			IgE
47	PS	RP	N	LFOA-	AR	ALMNV						
48	GV	P	P	KHQD	SHQE							
49	KN	Q-	TMC	WYC-	Q	Q	FP-	VP-	L	W-	D	
50	PST	STAPLWV	W	WY-	RHD							
51	WH	TSKHRQG	LIRKGP	DSRTQGH-	DEKQHT	H	RKQDT					
52	Q	DNT-	W	R	STRE-	A	FW					

Example 10

For this example the third-generation epitope sequences were determined in further 11 environmental allergens (Bosd2, Equcl, Gald4-mutant (with alanine substituted for glycine in position 102), Hevb8, Profillin1-AC, Profillin1-AT, Profillin2-AC, Profillin-birch pollen, Rag weed pollen5 and Vesv5), based on their structures sequences (SEQ ID NO: 12, 13, 15, 16, 17, 18, 19, 20, 21 and 22, respectively), their structures (1bj7.pdb, 1ew3.pdb, 1flu.pdb, 1g5u.pdb, 1prq.pdb, 1a0k.pdb, 1f2k.pdb, 1cqa.pdb, 1bbg.pdb, and 1qnx.pdb, respectively), and computer modelling of the epitope patterns that had been assembled in our database (shown in Table 8). Further, the epitope sequences of the four environmental allergens of example 9, Bet v1, Der f2, Der p2, and Phl p2, were redetermined.

The additional allergens arise from common sources of allergy: cows (Bos d2 which is a bovine member of the lipocalin family of allergens), horses (Equ C 1, a major horse allergen aslo of the lipocalin family), Hen egg white (Lysozyme Gal D 4), Latex (Hev b8, a profilin from Hevea Brasiliensis), Acanthamoeba castellanii (Profillin1-AC, a profilin isoform IA and Profillin2-AC, a profilin isoform II), Arabidosis thaliana (Profillin1-AT a cytoskeleton profilin), Birch (Profillin-birch pollen (Birch pollen profilin), Rag weed pollen5 (Ragweed pollen allergen V from Ambrosia trifida) and whasp venom (Ves v5 allergen from Vespula vulgaris venom).

The protein surface is scanned for epitope patterns matching the given "consensus" sequence of about 6-12 residues. First, residues on the protein surface that match the first residue of the consensus sequence are identified. Within a specified distance from each of these, residues on the protein surface that match the next residue of the consensus sequence are identified. This

procedure is repeated for the remaining residues of the consensus sequence. The method is further described under the paragraph "Methods" above and the program can be found in Appendixes.

5

The critical parameters used in this screening included:

- i) a maximal distance between the alfa-carbon atoms of subsequent amino acids,
- ii) a minimal accessibility of the amino acid of 20Å²,
- iii) the largest maximal distance between the most distinct amino acids should be less than 25Å
- iv) the best epitope were taken,
- v) the homology with the epitope pattern of interest was 100%

15

In this way a number of potential epitopes are identified. The epitopes are sorted according to total surface accessible area, and certain entries removed:

20

25

- a. Epitopes that contain the same protein surface residue more than once. These are artefacts generated by the described algorithm.
- b. Epitopes which are "too big", i.e. where a distance between any two residues in the epitope exceeds a given threshold.

30 The epitope sequences found were:

Bosd2:

35

Epi#01

L65, P155, P156, R17, R40, N37, Y39, R41, T67
L65, P155, P156, R17, R40, N37, Y39, R41, S52
L64, P155, P156, R17, R40, N37, Y39, R41, T54

5 Epi#02

T121, K150, S122, R17, P156, Y39, R41, R40
T121, K150, S122, R17, P156, Y56, R36, V30

Epi#03

10 L128, K130, H92, I7, Y76
L134, K130, H92, I7, Y76
L128, K130, H92, I91, Y76

Epi#04

15 R72, Y76, S50, Q73, K71, V69, I45
K71, Y76, S50, Q73, R72, V69, L80
K71, Y76, S50, Q73, R72, V69, I42

Epi#06

20 G14, P13, D47, S10, K11, P9
G14, P13, D47, S10, S94, P9
G14, P13, D47, C44, S10, P9

Epi#08

25 K71, E49, S50, V69, F82
K71, E49, S50, V79, F82

Epi#09

I7, S10, D8, E95, K119, N96, S122, T121
30 S10, I7, D8, E95, K11, N96, S122, T124

Epi#10

E15, T54, R41, T67, F55, R17, K119
E43, T54, R41, T67, F55, R17, K119
35 E31, T151, N153, C63, F55, R40, R41
E31, T151, N153, C154, F55, R41, R17

Epi#11

K26, I145, Q132, E143
40 K26, I145, Q132, E137
K26, I145, Q132, E129

Epi#12

Y105, E108
45 Y83, E81

Epi#15

N153, P156, D152, I149, T121, G120
R17, P156, D152, I149, T121, G120
50 N153, P156, D152, I149, R17, G14

Epi#18

R109, I110, G107, Y83, T85, E81, V69

R109, I110, G107, Y105, T85, E81, V69

5

Epi#19

E43, N46, S50, Q73, R72, K71

D47, N46, S50, Q73, R72, G75

E49, N46, S50, Q73, R72, K71

10 I45, N46, S50, Q73, R72, K71

Epi#20

V30, K28, P34, L57, L65, K58, D59, G32, D27

V30, K28, P34, L57, L64, K58, D59, G33, D27

15

Epi#22

D8, S10, D47, P13, E15

D8, S10, D47, P13, E43

D47, S10, D8, V93, E95

20 D8, S10, D47, C48, K71

Epi#23

K119, N96, E127, S122, L128, P125

K150, N147, E146, Y20, F123, P125

25 K11, N96, E127, S122, L128, P125

Epi#24

E129, K130, E126, P125, S122, L128, Q133

E126, K130, E129, P125, S122, R17, K119

30 E126, K130, E129, P125, T124, L128, Q133

Epi#25

R72, K71, I45, D47, N46

R72, K71, I45, E43, N46

35

Epi#27

D47, E49, E74, K71

D24, E143, E146, K150

D47, E43, E15, K119

40

Epi#28

L134, Q133, L128, E126, K130, F123, S122, K150

Q132, K130, E126, L128, F123, S122, K150

L65, D59, Q60, K58, E31, L57, G32, D27

45 G61, D59, Q60, K58, E31, K28, G32, D27

Epi#29

V69, K71, L80, R72, E74

I45, K71, L80, R72, E74

50 G61, Q60, L64, F55, E68

Epi#30

G120, N96, S94, H92, K130, L128, P125
I91, I7, S94, H92, K130, L128, P125

5

Epi#33

K130, F123, P125, S122, K150
K71, Y76, P9, S10, S94, K119

10 Epi#34

I7, P9, S10, G14, R17, T121, S122
I45, P13, S10, G14, R41, Y39, P156

Epi#37

15 T67, V69, L80, K71, Y76
P156, R40, L65, K58, D59
P155, R40, L65, K58, N153

Epi#38

20 L80, G84, E108, R109, N25, P141, S136

Epi#39

E137, R138, P141, G139, L134
E31, L57, R36, P34, G84, L80

25

Epi#40

R17, G120, T121, K150, S122
R17, G120, T121, K150, T151

30 Epi#41

P34, Y83, L80, V69, S52
P34, Y83, L80, V79, S50

Epi#42

35 L128, P125, S122, G120, R17, R41
L128, P125, S122, G120, R17, R40

Epi#44

S10, D47, P9, Y76, S50, V69, T67
40 I45, D47, P9, Y76, S50, V69, T67

Epi#45

D27, P34, F82, Y105, R109, D106, G107
D59, P34, F82, Y105, R109, D106, G107
45 K58, P34, F82, Y105, R109, D106, G107
D27, P34, F82, Y105, R109, D106, G84

Epi#46

Y39, R41, R40, P155, C63, Q60
50 Y20, R17, R40, P155, C63, Q60

Epi#47

L128, E126, E129, L134, R138, Q133, N142, P141, S136
V69, E81, E68, I42, R41, F55, N37, R40, P156
5 V69, E43, E15, I42, R41, F55, N37, R40, P156
S122, E127, E129, L134, R138, Q133, N142, P141, S136

Epi#48

E43, D47, P13, P9, V93
10 S10, D47, P9, P13, G14
E43, D47, P13, P9, V90
E49, D47, P13, P9, V93

15

Equcl:

Epi#02

L66, N68, A65, F90, S69, Y72, R64, V89
20 A65, R64, S31, F28, S112, Y123, R110, V108
L179, R180, Q178, F177, P143, Y38, R141, V145
L66, R64, S31, F28, S112, Y123, R110, V125
L66, N68, A65, F90, S69, Y72, R64, V62

25 Epi#03

K32, A65, I63, Y72

Epi#05

G35, A65, S69, T93, G97, R26, S112, Y123
30 G35, A65, S69, T93, G97, R26, S112, I25

Epi#07

G97, T93, S70, D91, S100, R110, V125, P132, D128

35 Epi#08

K129, D130, F127, V108, F90
K129, D130, F127, V108, F109
K129, D130, F127, V125, F136
K129, D130, F127, V125, F133

40

Epi#10

E48, N53, N80, T77, C83, F177, R175, K172
E82, N80, N53, T77, C83, F177, G181, R180
E52, N53, N80, T77, C83, F177, R175, K172

45

Epi#11

F133, K47, I167, Q158, V163, E165

Epi#12

50 Y38, E142

214

Y38, E36
Y139, E142

Epi#13
5 K129, P132, D45, I167, Q158, G161
R131, P132, D45, I167, K164, G161

Epi#16
P87, Y72, R64, S70, S69, D67, A65, N68

10 Epi#17
A65, S31, R64, S34

Epi#18
15 R64, S31, I30, A65, S34, L66, N68, S69

Epi#19
E82, N80, C83, Q178, R175, K172

20 Epi#22
D130, P132, D128, Y106, K129

Epi#23
D144, K150, E148, P147, S146, E151, K155

25 Epi#25
R160, K159, I156, E151, E148

Epi#27
30 E118, E142, D144, K172
E36, E142, D144, K172

Epi#28
I173, D174, Q178, L179, E85, C83, F177, G181, R180
35 I173, D174, Q178, L179, E85, C83, F177, P143, D144

Epi#29
G181, Q178, L179, R180, E36
G181, Q178, L179, R180, E85

40 Epi#30
I30, N27, S112, H119, I121, I25, V23

Epi#31
45 L122, R110, N27, R26, F28, I30, D96
L124, R110, N27, R26, F28, I30, D96

Epi#33
H119, Y38, V62, S34, S31, R64

50

215

Epi#34

V62, P87, M88, V89, R64, S31, S34

Epi#37

5 P87, V89, L66, R64, D67

Epi#40

R64, L66, A65, Y72, S34

R64, L66, A65, Y72, S69

10

Epi#41

P132, Y106, L101, V89, S100

P132, Y106, L101, V89, S70

15 Epi#44

V46, R131, D128, P132, Y106, S100, V89, P87

Epi#45

K129, P132, F127, Y106, N102, D91, V89

20 K129, P132, F127, Y106, N102, D104, G105

Epi#47

S146, E148, E152, V23, R26, A24, N27, R110, S112

V23, E115, E118, N116, R26, F28, N27, R110, S112

25

Gald4:

30

Epi#01

L75, N65, P70, R73, R61, N59, Y53, R45, T47

L75, N65, P70, R68, R61, N59, Y53, R45, T47

35 Epi#02

A90, N77, L75, R73, P70, R61, R68

A122, R125, Q121, T118, R114, R112

Epi#04

40 R21, Y20, S24, Q121, R125, R128, L129

R21, Y20, S24, Q121, R125, R128, G126

Epi#05

G16, A10, R128, G126, A122, T118, G117

45 G4, A10, R128, G126, A122, T118, G117

Epi#06

G67, P79, D66, R61, R73, P70

G67, N65, D66, S72, R73, P70

50 G49, N46, D48, R61, R73, P70

Epi#07
G71, T69, D66, S72, R73, P70, D48
G67, T69, D66, S72, R73, P70, D48

5 Epi#08
K1, D87, S86, V2, F38
K1, D87, S86, V2, F3

10 Epi#09

Epi#10
E7, A11, R14, A10, C6, F3, R5, R125
D87, A11, R14, A10, C6, F3, R5, R125
15 T47, N46, N44, S36, F34, R114, R112
D18, A10, R14, A11, C6, F3, R5, R125
T118, N113, R112, A110, F34, R114, K116

Epi#11
20 L129, I124, Q121, V120, D119

Epi#12
Y53, E35

25 Epi#15
R73, P70, D66, I78, A82
R73, P70, D66, I78, A90

Epi#17
30 A102, S100, R21, S24

Epi#18
R112, N113, R114, F34, V109, A107, A102, N103
N113, R112, R114, F34, V109, A107, N103, S100

35 Epi#19
D18, N19, S24, Q121, R125, L129
D18, N19, S24, Q121, R125, G126

40 Epi#22
D48, P70, D66, W63, W62
D66, P70, D48, T69, W62
D48, P70, D66, W63, K97

45 Epi#23
R45, N44, E35, N39, Q41, A42
R45, N44, E35, Y53, Q41, A42

Epi#25
50 R128, R125, W123, D119, N27

217

R128, R125, W123, D119, V120

Epi#26

W62, S72, W63, P79, A82, D87

5 W62, S72, W63, P79, G67, D66

Epi#28

G117, D119, Q121, I124, E7, C6, F3, A11, R14

A122, D119, Q121, I124, E7, C6, F3, A11, R14

10

Epi#29

G126, R125, L129, R128, E7

G16, R14, L129, R128, E7

15 Epi#30

I124, L129, A10, H15, I88, L84

I124, L129, A11, H15, I88, L84

Epi#31

20 L75, R73, N65, R61, W62, I98, D101

L75, R73, N74, R61, W62, I98, D101

Epi#33

Q41, F38, V2, S86, S85, K1

25 Q41, F38, V2, S36, A110, R114

Epi#34

W63, W62, T69, G71, R73, S72, P70

W62, W63, S72, L75, R73, T69, P70

30

Epi#36

A110, A107, A102, S100, K96, A90, A82

Epi#37

35 A10, R128, L129, R14, D18

A10, R128, L129, K13, N19

Epi#40

R128, L129, A11, T89, A90, S85

40 R14, L129, A11, T89, A90, S85

Epi#41

Y53, L84, S81

Y53, L84, S86

45

Epi#42

P79, S81, N65, P70, R61, R73

P79, S81, N65, P70, R61, R68

50 Epi#44

L129, R14, D18, Y20, S24, V120, T118

L129, R14, D18, Y23, S24, V120, T118

Epi#46

5 L75, R61, R73, P70, N65, G67

L75, R73, R61, P70, N65, A82

L75, R61, R68, P70, N65, G67

Epi#47

10 S72, G71, R68, N65, R61, L75, N77, R73, P70

G67, S72, R68, N65, R61, L75, N77, R73, P70

Epi#49

D87, L84, Q41, Q57, Y53, T43, N44

15 D87, L84, Q57, Q41, Y53, T43, N46

D87, L84, Q41, Q57, Y53, T43, N39

Epi#50

R73, W62, W63, P79, S81

20 R73, W63, W62, S72, P70

Epi#51

D18, H15, K13, R14, L129, R125, W123

25 Epi#52

F34, A110, R112, R114, W111, N27, Q121

F3, A11, E7, R5, W123, D119, Q121

W123, A122, T118, R114, W111, N27, Q121

30

Hevb8:

Epi#01

L20, P109, P112, K86, R84, N116, Y125, Q129, T111

35 L110, P109, P112, K86, R84, N116, Y125, Q129, T111

Epi#02

A48, K43, Q41, F42, T70, Y72, R84, V74

T21, R19, P109, P112, R84, V74

40 A49, K43, Q41, F42, T70, Y72, R84, V74

Epi#03

L65, K86, I75, Y72

45 Epi#05

G30, A48, L60, P62, G58, T63, H66, G69

G58, A61, R84, P112, G113, T111, S89, G88

G80, A81, F54, P79, G58, T63, H66, G69

G77, A81, F54, P79, G58, T63, H66, G69

50

Epi#06
G58, P79, D55, S59, K52, P57
G80, P79, D55, S59, K52, P57
G77, P79, D55, S59, K52, P57

5
Epi#07
G17, T5, S2, D16, R19, P109, D107

Epi#08
10 K52, D45, S44, A49, H66, F42

Epi#10
E78, A81, R96, F54, G58, K52
D55, A81, R96, F54, G80, K52

15
Epi#11
F54, L60, I83, Q76, V82, E78

Epi#12
20 Y106, E108

Epi#13
H66, L65, P62, T63, A61, P57, A81, P79, G58
H66, L65, P62, T63, A61, P57, A81, P79, G80
25 H66, L60, P62, T63, A61, P57, A81, P79, G77

Epi#15
R19, P109, D107, I105, K86, G88

30 Epi#18
R19, G17, P109, S89

Epi#22
D29, S44, D45, A48, K52
35 D29, M51, D55, P79, E56
D45, M51, D55, P79, E78
D29, S44, D45, A49, K52
D45, M51, D55, P79, E56
D29, M51, D55, P57, E78
40 D29, M51, D55, P57, E56
D45, M51, D55, P57, K52
D45, M51, D55, P57, E78

Epi#24
45 D55, K52, E56, P79, F54, E78, Q76
D45, K52, E56, P57, F54, E78, Q76

Epi#25
R84, K86, I105, D107, E108
50 R96, H28, I26, D29, V3

Epi#26

W33, S2, W3, V32, G30, D29

5 Epi#27

D53, E56, D55, K52

Epi#28

V32, Q41, K43, E46, K52, F54, P57, D55

10 G69, Q41, K43, E46, K52, F54, P57, D55

Epi#29

G130, Q99, L127, R96, E78

L127, Q99, L131, R96, E78

15 G98, Q99, L127, R96, E78

Epi#30

G69, L67, A49, H66, K71, L65, P62

G80, M51, A48, H28, Q99, L127, L131

20

Epi#33

Q41, F42, V32, S31, S44, K43

Q41, F42, V47, S44, A48, K52

Q41, F42, V47, S44, A49, K52

25

Epi#34

I105, P112, S89, L110, R19, T21, S37

I105, P112, T111, L20, R19, T21, S37

30 Epi#37

T63, A49, L60, K52, D55

P62, V74, L60, K52, D45

P62, A61, L60, K52, D55

35 Epi#38

G77, E78, R96, V82, R84, N116, P112, S89

Epi#39

A48, E46, H66, T63, P62, G58, L60

40 A49, E46, H66, T63, P62, G58, L60

Epi#40

R19, L110, G113, T111, P109, S89

R19, L110, G113, T111, P112, S89

45

Epi#41

P62, L65, V47, S44

P109, Y106, L110, S89

P112, Y106, L110, S8

50

221

Epi#44

L20, R19, D16, W3, Y6, S2, G17, P109
L110, R19, D16, W3, Y6, S2, G17, P109

5 Epi#45

K52, P57, F54, R96, D124, L127
D55, P79, F54, R96, D124, L131

Epi#47

10 I75, G77, E78, V82, R84, N116, P112, S89
I75, G77, E78, I83, R84, N116, P112, P109

Epi#48

E78, Q76, P79, P57, G58
15 E78, Q76, P79, P57, G80
E78, Q76, P79, P57, G77

Epi#50

D9, W3, W33, S2, T5
20 D16, W3, W33, S2, T5

Epi#51

R19, H18, E108, S89, K87, K71, H66
R19, H18, E108, D107, K87, K71, H66
25

Profillin1-AC:

Epi#01

30 L116, N111, P106, K80, K81, N101, S83, Q105, T108
L116, N111, P106, K80, K81, N101, Y100, Q105, S83

Epi#02

T44, N51, P54, R56, T69, Y78, R71, V68
35 L24, K93, S92, R75, S76, Y78, R71, R56

Epi#03

L24, K93, I121, Y119
L24, K90, I121, Y119
40

Epi#04

K80, Y100, S83, Q105, K103, N101, G82
K80, Y100, S83, Q105, K103, T17, G12
K80, Y100, S83, Q105, K103, T17, G14
45

Epi#05

G34, A33, A36, T38, G64, A63, H66, V68
G34, A33, S32, T17, G12, T4, S1, Y5

50 Epi#06

- A46, N50, D53, R56, A57, P54
A52, N50, D53, R56, A57, P54
A72, N50, D53, R56, A57, P54
A57, P54, D53, S47, Q43, P39
- 5 Epi#07
G64, T38, D61, S58, R56, A57, P54, D53
G64, T38, D61, S58, R56, A52, P54, D53
- 10 Epi#08
K103, E102, G82, V68, H66, F60
K81, E102, G82, V68, H66, F60
- Epi#09
15 L24, S47, D53, A57, V68, R71, L70, R56, N51, N50, R75
L24, S47, D53, A57, V68, R71, L70, R56, N51, T44, T38
- Epi#10
D74, N50, N51, T44, F60, R56, R71
20 D53, N50, N51, T44, F60, R56, R71
- Epi#11
F125, K93, I121, Q123, D118
F125, K90, I121, Q123, D118
25 F49, K90, I121, Q123, D118
- Epi#12
Y119, E114
Y100, E102
- 30 Epi#13
A57, R56, P54, T44, A40, P39, A36, G64, Y67
S58, A57, P54, T44, A40, P39, A36, G64, Y67
- 35 Epi#15
N51, P54, D53, I55, R56, A57
R56, P54, D53, I55, T69, A57
R56, P54, D53, I55, T44, A40
- 40 Epi#16
Q105, P106, Y100, G14, Q18, S32, A36, A33, D7
Q105, P106, Y100, G14, Q18, S32, A36, A63, D61
- Epi#17
45 A110, S76, R75, S92
A72, S76, R75, S92
- Epi#18
N51, N50, R75, S92, L24, S47, T44, P39, N27
50 N51, N50, R75, S92, L24, T28, T38, P39, N27

- Epi#22
D53, S47, D25, L24, K93
D53, S58, D61, V68, K81
- 5 Epi#23
K103, N101, E102, S83, Q105, P106
K103, N101, E102, S83, Q105, A84
- 10 Epi#24
E114, K115, A110, P106, S83, E102, K103
D53, G59, A57, P54, R56, L70, K80
E102, K103, A15, P106, S83, A84, Q105
- 15 Epi#25
R71, R56, I55, D53, N50
R71, R56, I55, D53, N51
- Epi#28
- 20 I104, Q105, K103, E102, K81, S83, K80
G107, Q105, K103, E102, K81, G82, K80
A84, Q105, K103, E102, K81, S83, K80
A110, Q105, K103, E102, K81, S83, K80
- 25 Epi#29
I121, K115, L116, E114
V112, K115, L116, E114
- Epi#30
- 30 G59, I55, S58, H66, K80, L70, V68
G59, I55, S58, H66, K80, P106, V99
- Epi#33
- 35 K80, Y78, V68, S58, A57, R56
K81, Y67, V68, S58, A57, R56
- Epi#34
I55, P54, S58, V68, R71, Y78, P106
W29, W2, T4, V11, G12, Y5, S1
- 40 Epi#36
A63, A36, A33, V11, G14, Y100, S83, Q105, K103, P106, A110, A15
A63, A36, A33, V11, G14, Y100, T108, Q105, K103, P106, A15, A110
- 45 Epi#37
A57, R56, L70, R71, Y78
A57, V68, L70, R56, D53
Y78, R71, L70, R56, N51
P54, R56, L70, R71, D73
- 50 T69, R71, L70, R56, D53

Epi#38

G82, E102, A84, V99, N101, P106, S83

5 Epi#40

R71, L70, A72, Y78, K80, S83

R71, L70, G59, T69, K81, S83

R56, L70, A72, T69, K81, S83

10 Epi#41

P106, Y78, L70, V68, S58

Epi#42

P54, S47, N51, R56, R71

15 P54, S58, G59, R56, R71

Epi#44

S83, Q105, P106, Y78, A110, G107, T108

V68, R71, D73, Y78, A110, G107, T108

20 L70, R71, D73, Y78, A110, V112, T108

L70, R71, D73, Y78, A110, G107, P106

Epi#45

K81, H66, F60, R56, D53, G59

25 K80, H66, F60, R56, D53, G59

D61, H66, F60, R56, D53, G59

Epi#46

L70, R71, R56, P54, N51, A52

30 L70, R71, R56, P54, N51, A72

V68, R71, R56, P54, N51, A46

Y78, R71, R56, P54, G59, A57

Epi#47

35 V68, A57, R56, L70, R71, A52, N51, P54, S58

S58, A57, R56, L70, R71, A72, N51, P54, S47

Epi#49

D25, L24, Q43, Q41, T44, N51

40 D25, L24, Q43, Q41, T38, N27

Epi#50

D7, W2, W29, S1, T4

D7, Y5, W2, W29, S1

45

Epi#51

K80, H66, D61, T44, P39, T28, W29

K80, H66, D61, T38, P39, T28, W29

Profillin1-AT:**Epi#01**

P109, P89, K86, R84, N116, Y106, Q114, T111

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Epi#02

L42, K43, Q45, F66, T63, Y72, R84, V74

L42, K43, Q45, F66, T63, Y72, R84, V82

10 **Epi#03**

K96, I127, Y125

K86, I75, Y72

Epi#05

15 G77, A81, F54, P57, G58, A61, T63, V74

G58, A61, F59, P57, G77, A81, T97, G80

G80, A81, F54, P57, G58, A61, T63, Y72

Epi#06

20 G17, P109, D107, T21, K38, P40

G112, P109, D107, T21, K38, P40

G88, P89, D107, T21, K38, P40

Epi#08

25 K52, E55, G58, V74, F66

K51, E55, G58, A61, F59

Epi#09

D29, D48, K52, F59, A61, T63

30 D29, D48, K51, F59, A61, T63

Epi#10

E108, T111, N18, T21, F39, G68, K71

E108, T111, N18, T21, F105, G112, K86

35

Epi#11

F105, K86, I75, Q76, V82, E78

F66, K43, I47, Q28, V32, D29

F59, K52, I47, Q28, V32, D29

40

Epi#12

Y125, E130

Y125, E128

45 **Epi#15**

K43, P44, D29, I47, K52, G58

K43, P44, D48, I47, Q45, G49

K43, P44, D29, I47, K51, G80

50 **Epi#20**

226

K38, P40, F39, L42, K43, D48, G30, D29
K51, P57, F59, L60, K52, D48, G30, D29

Epi#22

5 D48, P44, D29, V32, W33
D48, P44, D29, V32, W3

Epi#24

D29, K51, E56, P57, F59, E55, Q79
10 D48, K52, E55, P57, F59, E56, Q79

Epi#25

R121, K95, I83, D53, E55
R121, K95, I83, E78, V82

15

Epi#26

W33, S2, W3, V32, G30, D29

Epi#27

20 E128, E130, D124, K96
E130, E128, D124, K95

Epi#28

I75, Q76, E78, Q79, P57, K51
25 A61, Q76, E78, Q79, P57, K52
V32, D29, Q99, E130, I127, S129, D124
V32, D29, Q99, I127, E128, S129, D124

Epi#29

30 V32, Q41, L42, F66, E70
G69, Q41, L42, F66, E70
G68, Q41, L42, F66, E70

Epi#30

35 G17, N18, H19, Q114, L117, V15
G17, M110, H19, Q114, L117, V15
G113, M110, H19, Q114, L117, V15

Epi#33

40 Q41, F39, P40, S36, A37, K38

Epi#34

V74, P62, M73, G88, P89, Y106, T111

45 Epi#37

T111, V15, L117, R121, Y125
T111, V15, L117, R121, D124

Epi#39

50 A81, E55, P57, G58, L60

227

A81, E78, P57, G58, L60

Epi#40

R121, L117, G112, Y106, P109, T111

5 R121, L117, G112, Y106, P89, T111

Epi#41

Y125, L131, S129

10 Epi#44

I75, R84, Y72, A61, G58, P62

I75, R84, Y72, A61, V74, T63

Epi#45

15 K38, P40, F105, Y106, N18, D14, G17

K38, P40, F105, Y106, N18, D107, G88

K38, P40, F105, Y106, N18, D14, V15

Epi#48

20 E16, H19, P109, P89, G88

E16, H19, P109, P89, G112

Epi#49

D124, L131, Q99, Q28, T97, N98

25 D124, L131, Q99, Q28, T97, K96

Epi#50

D9, Y6, W3, W33, S2

D9, W3, W33, S2, S5

30 D9, W3, W33, V32, S31

Epi#51

D14, H19, E108, T111, L117, R121, H10

D107, H19, E16, Q114, L117, R121, H10

35 D14, H19, D107, T21, K38, Q35, W33

Profillin2-AC:

40 Epi#01

L116, N111, P106, K80, K81, N101, S83, Q105, T108

L116, N111, P106, K80, K81, N101, S83, Q105, T108

Epi#02

45 T53, N58, S57, R56, T69, Y67, R66, V68

T53, K50, A52, R56, T69, Y67, R66, V68

T53, K50, A72, R56, T69, Y67, R66, V68

Epi#03

50 L116, K115, I121, Y119

Epi#04

K81, Y100, S83, Q105, K103, T17, G12
K80, Y100, S83, Q105, K103, A84, G82
5 K81, Y100, S83, Q105, K103, T17, G14
K80, Y100, S83, Q105, K103, N101, I104
K81, Y100, S83, Q105, K103, A15, G107

Epi#06

10 A54, N47, D25, T28, A36, P39
A40, N27, D25, T28, A36, P39
A44, N47, D25, T28, A36, P39
G34, A33, D7, T31, A36, P39
A43, N47, D25, T28, A36, P39

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Epi#08

K103, E102, G82, V68, F60
K103, E102, G82, V68, F60
K81, E102, G82, V68, F60

20

Epi#10

T53, N58, R56, S57, F60, R66, K81
E61, N58, R56, S57, F60, R66, K80

25

Epi#11

F125, K93, I121, Q105, E102
F125, K93, I121, Q123, D118

Epi#12

30 Y100, E102
Y119, E114

Epi#13

A52, A44, P39, A43, H24, S92, G124, Y119
35 A46, A44, P39, A43, H24, S92, G124, Y119

Epi#15

K103, P106, D118, I121, K93, G124
K103, P106, D118, I121, Q105, G107
40 K103, P106, D118, I121, Q123, G122

Epi#16

Q105, P106, Y78, R71, S57, N58, A54, A44, D51
Q105, P106, Y78, R71, R56, D51, D74, A52, N47

45

Epi#18

R66, N58, R56, S57, V68, G82, S83, E102, N101
R66, N58, R56, S57, V68, G82, S83, P106, N101

50

Epi#22

D74, A52, D51, T53, K50
D25, A44, D51, T53, K50
D74, A46, D51, T53, K50
D74, A72, D51, T53, K50

5

Epi#23
K103, N101, E102, S83, Q105, P106
K103, N101, E102, S83, Q105, A84

10 Epi#24

D74, K81, A84, P106, S83, E102, K103
D74, K81, E102, P106, T108, A15, K103

Epi#25

15 R66, K81, E102, N101

Epi#28

I121, D118, Q105, K103, E102, K81, G82, D74
G107, D118, Q105, K103, E102, K81, G82, D74
20 G122, D118, Q105, K103, E102, K81, G82, D74

Epi#29

I121, K115, L116, E114
V112, K115, L116, E114

25

Epi#30

I55, N47, A44, H24, K93, I121, L116
I55, N47, A43, H24, K93, I121, L116

30 Epi#31

R56, N58, R66, F60, V68, I55, D51
R66, N58, R56, F60, V68, I55, D51

Epi#33

35 K115, Y119, P106, S83, A84, K103
Q123, Y119, P106, S83, A84, K103
K81, Y67, V68, S57, A54, R56
K80, Y78, V68, S57, A54, R56

40 Epi#34

W29, W2, T8, V11, G12, T4, S1
W29, W2, T4, G12, G14, T13, T8

Epi#37

45 T108, V112, L116, K115, Y119
T108, A110, L116, K115, N111
T13, V112, L116, K115, D118
P106, A110, L116, K115, N111

50 Epi#38

230

G64, E61, A40, V37, N27, P39, S38
G82, E102, A84, V99, N101, P106, S83

Epi#39

5 A110, E114, T108, P106, G122, L116

Epi#40

G14, G12, T17, K103, S83
R56, A52, T53, A54, S57
10 R66, A63, T65, K81, S83
R56, A72, T53, A54, S57
R56, G59, T53, A54, S57
R66, G64, Y67, K81, S83

15 Epi#42

P106, S83, G82, R75, R71

Epi#44

S1, Q3, D7, W2, Y5, S32, G12, T8
20 S1, Q3, D7, W2, Y5, A30, A36, P39
S1, Q3, D7, W2, Y5, S32, V11, T8
S1, Q3, D7, W2, Y5, S32, G12, T4
S1, Q3, D7, W2, Y5, A30, A33, T31
S1, Q3, D7, W2, Y5, A30, A36, T28
25 S1, Q3, D7, W2, Y5, S32, G12, T13
S1, Q3, D7, W2, Y5, S32, G34, T31

Epi#45

K93, H24, F49, R75, D74, G82
30 D25, H24, F49, R75, D74, G82

Epi#47

A36, G64, E61, A40, A44, A54, N58, R56, S57

35 Epi#50

D7, Y5, W2, T8, S1
D7, W2, W29, T28, P39

Epi#51

40 K90, H24, K93, D25, P39, T28, W29
T91, H24, K93, D25, P39, T28, W29

Profillin-brich pollen:

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Epi#01

L124, N118, P114, K88, K73, H68, Y74, R86, T95

Epi#02

50 T113, N118, Q116, P114, R86, V76
T50, K54, L62, T65, Y74, R86, V84

Epi#03

L133, K98, I129, Y127

5 Epi#04

S40, Q43, K45, T50, G32

S40, Q43, K45, T50, G51

S40, Q43, K45, T50, I49

10 Epi#05

G82, A81, A83, P59, G60, A63, T65, V76

G82, A83, A81, P59, G60, A63, H61, V76

G79, A81, A83, P59, G60, A63, T65, V76

15 Epi#06

G70, P46, D31, T50, K54, P59

A81, P59, D55, T50, Q47, P46

G32, P46, D31, T50, K45, P42

G51, P46, D31, T50, K54, P59

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Epi#08

A81, E57, G60, A63, H61, F56

A81, E57, G60, V76, H68, F44

K54, E57, G60, A63, H61, F56

25

Epi#11

F56, K98, I85, Q78, V84, E122

F56, K98, I27, Q37, V34, D31

F56, K97, I85, Q78, V84, E80

30

Epi#12

Y6, E9

Y127, E122

35 Epi#13

H68, L62, P64, T65, A63, P59, A81, G82, G79

H61, L62, P64, T65, A63, P59, A81, G79, F56

H68, L62, P64, T65, A63, P59, A83, G79, G60

40 Epi#15

K45, P46, D31, I49, Q47, G32

K45, P46, D31, I49, K54, G60

K45, P46, D31, I49, K54, G82

K45, P46, D31, I49, T50, G51

45

Epi#16

Q116, P114, Y108, M12, S39, S40, A23, A24, D8

Q116, P114, Y108, M12, Q37, S40, A23, A24, D8

R86, P114, Y108, M12, S39, S40, A23, A24, D8

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Epi#22

D126, L133, D130, Y127, E122

D130, L124, D126, Y127, E122

D130, L128, D126, Y127, E122

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Epi#23

R123, N118, E122, L124, L11, A23

R123, N118, E122, L124, L11, A36

R123, N118, E122, L124, L11, A24

10

Epi#24

E109, G90, E110, P114, R86, E80, Q78

E57, K54, E58, P59, F56, A81, Q78

E58, G60, E57, P59, F56, E80, Q78

15

Epi#25

R86, K88, I107, E109, E110

R86, K88, I77, E80, V84

R86, K88, I107, E109, V112

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Epi#27

57, E58, D55, K54

D55, E57, E58, K54

25 Epi#28

V34, D31, Q101, K98, E122, L128, Q131, G132, D130

I129, D126, Q131, L128, E122, K98, Q101, G100, D130

I72, H68, Q47, F44, E48, K45, Q43, G70, K73

I72, H68, Q47, I49, E48, K45, Q43, G71, K73

30

Epi#29

I129, Q101, L128, R123, E122

G132, Q131, L128, R123, E122

35 Epi#30

I77, M75, A63, H61, P59, L62, P64

G90, M75, A63, H61, K54, L62, P64

Epi#33

40 Q116, Y108, P111, S91, K89

K88, Y108, P111, S91, K8

Epi#34

V76, P64, M75, L62, G51, T50, P46

45 I27, W35, S33, V34, G32, T50, P46

V76, P64, T65, L62, G51, T50, P46

Epi#35

A24, L22, A23, S39, M12, I107

50 A23, L11, A36, S39, M12, I10

233

Epi#37
Y127, R123, L124, K97, N118
Y108, A23, L11, R123, Y127
5 A23, A24, L11, R123, Y127

Epi#39
A81, E57, H61, T65, P64, G60, L62
A81, E58, H61, T65, P64, G60, L62
10

Epi#40
R123, L11, A23, Y108, P111, S91
R123, L11, A24, Y108, P111, T113

15 Epi#41
P111, Y108, L22, V112, S91
P114, Y108, L22, V112, S91

Epi#43
20 I27, W35, A36, L11, Q37, S39, M12, I107, T95

Epi#44
I77, R86, P114, Y108, S91, V112, P111
V120, Q116, P114, Y108, S91, V112, P111
25 L22, Q116, P114, Y108, S91, V112, T113
L22, Q116, P114, Y108, A23, V112, P111

Epi#47
I129, Y127, E122, M119, R123, L124, N118, R86, P114
30 L133, Y127, E122, M119, R123, L124, N118, R86, P114

Epi#48
E122, Q116, P114, P111, V112
S91, K88, P114, P111, V112
35

Epi#50
H10, Y6, W3, S2, T5
H10, Y6, W3, T5, S39

40 Epi#51
K73, H68, K45, Q47, P46, S33, W35
Q101, H30, D31, T50, K45, Q47, H68

45 Rag weed pollen5:

Epi#03
L4, K37, A33, I34, Y17
L4, K37, A33, I34, Y29
50

234

Epi#05

A33, N36, T40, G3, S20, L4
A33, N38, T40, G3, S20, Y25
A33, N36, G3, T40, S20, I22

5

Epi#06

A33, N36, D2, C19, K24, P21
A33, N38, D2, S20, K24, P21

10 Epi#09

I22, L4, D2, N38, D1, K37, A33, N36, T40
T9, G15, E7, V14, D30, K32, N36, T40, L4
T9, G15, E7, V14, D30, K32, N38, N36, L4

15 Epi#12

Y17, E7
Y6, E7

Epi#20

20 V27, K24, P21, L4, K37, D2, G3, D1
V27, K24, P21, L4, N36, D2, G3, D1

Epi#22

D1, D2, L4, K37
25 D1, D2, P21, K24
D2, L4, T40, D1

Epi#23

N10, E7, Y6, L4, P21

30

Epi#25

K32, I34, D30, V14
K37, I34, D30, V14
K16, I34, D30, V14

35

Epi#33

K32, Y17, V27, S20, K24
K16, Y6, P21, S20, K24

40 Epi#34

I22, P21, S20, V27, G12, Y17, T9
I22, P21, S20, V27, G12, Y29, S31

Epi#40

45 G12, G15, Y29, K37, T40
G15, G12, Y17, K16, T9
G12, G15, Y29, K32, S31

Epi#41

50 P21, Y6, L4, S20

Epi#44

L4, D2, P21, Y25, S20, V27, T40

L4, D2, P21, Y25, S20, G3, T40

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Vesv5:

Epi#01

10 L59, P67, P65, K143, K144, N64, Y140, R62, T61

L59, P67, P70, R57, K204, N73, Y201, Q202, T203

L59, P67, P69, R57, K72, N73, Y201, Q202, T203

L152, N149, P142, K145, K143, N64, Y140, R62, T61

15 Epi#02

L9, K7, Q108, P191, Y107, R102, V13

L9, K7, Q108, S192, Y107, R102, V13

Epi#03

20 L9, K7, A105, I6, Y3

Epi#04

K106, Y107, S192, Q108, K7, A105, I6

K106, Y107, S192, Q108, K7, V13, G12

25

Epi#05

G58, A56, R57, P69, G66, R62, T61, L59

G58, A56, R57, P69, G63, R62, T61, L59

30 Epi#06

G66, N64, D139, R62, K138, P67

G66, N64, D139, R62, K138, P65

G63, N64, D139, R62, K138, P67

35 Epi#08

K145, E199, S147, F151

K196, E198, S147, F151

K144, E199, S147, F151

40 Epi#09

L152, D150, S147, K144, N64, T61, L59

L152, D150, D139, K153, F151, S147, N197

D139, N64, R62, D135, K153, F151, S147, N197

45 Epi#10

E199, N197, N194, S147, F151, G148, K143

E199, N197, N194, S147, F151, G148, K196

E199, N197, N194, S147, F151, G148, K145

50 Epi#11

236

K179, I176, Q177, V30, E178
K29, I176, Q177, V30, E178

Epi#12

5 Y201, E199

Epi#13

S147, L200, P142, T203, A56, P70, L59, P67, G66
S147, L200, P142, T203, A56, P69, L59, P67, G58
10 S147, L200, P142, T203, A56, P70, L59, P67, G63
S147, L200, P142, T203, A56, P69, L59, P67, Y140

Epi#15

K106, P191, D103, I6, K5, A105
15 K106, P191, D103, I6, K7, G12

Epi#16

R57, P70, Y201, M74, Q53, N76, D50, A56, N73
R57, P69, Y201, M74, Q53, N76, D50, A56, N73
20 Q108, P191, Y107, R102, Q111, S192, D103, A105, N2

Epi#18

R57, L59, T61, P67, N64
R57, L59, T61, P65, N64
25

Epi#19

E167, N164, S192, Q108, R102, K7
E198, N194, S192, Q108, R102, K7
D103, T100, C8, Q108, R102, K7
30

Epi#22

L9, D103, T100, K10
A105, D103, L9, K7
D50, L45, D43, T37, K38
35 S147, D150, L152, K153

Epi#23

K196, N197, E199, N164, Q202, P70
K145, N197, E199, N164, Q202, P69
40

Epi#24

E198, K196, E199, P142, T203, P69, K143
E198, K145, E199, P142, T203, P70, K204
E198, K196, E199, P142, T203, P70, K72
45 E198, K145, E199, P142, F146, F151, K196

Epi#25

R57, K54, D50, N76
R57, K54, D50, E47
50

Epi#27

D43, E40, D125, K122

D50, E47, D43, K38

5 Epi#28

Q202, E199, K196, F151, S147, K144

Q202, E199, K196, F195, S147, K145

Epi#29

10 G58, R57, L59, R62, E136

G148, K145, L200, F195, E199

G148, K145, L200, F195, E198

Epi#33

15 K23, Y19, P24, S21, A16, K18

K23, Y34, P24, S21, A16, R102

Epi#34

I176, W180, T116, L115, G117, T119, S118

20 V31, P24, S21, L22, G35, Y34, T37

Epi#37

P69, R57, L59, K54, D50

P70, R57, L59, R62, D135

25 A56, R57, L59, R62, N64

P69, R57, L59, R62, D139

Epi#39

E199, L200, T203, P70, G58, L59

30 E198, L200, T203, P69, G58, L59

Epi#40

R57, L59, G58, T203, P69, T61

R57, L59, A56, Y201, K204, T203

35 R57, L59, A56, Y201, K72, T203

Epi#41

P24, Y19, L22, S21

P24, Y34, L36, S33

40

Epi#42

P191, S192, Q111, H98, R102, Q108

Epi#44

45 L59, R57, P70, Y201, A56, G58, T61

L59, R57, P69, Y201, A56, G58, T203

L59, R57, P70, Y201, A56, G58, P67

Epi#45

50 K153, H156, F151, Y140, N149, D150, L152

238

D135, H156, F151, Y140, N141, D150, L152
K143, P142, F146, Y140, N149, D150, L152

Epi#47

5 G58, L59, R57, M74, A56, Q202, N73, P70, P69
G148, Y140, R62, L59, R57, A56, N73, P70, P67
G66, G63, R62, L59, R57, A56, N73, P70, P67
G155, E136, R62, L59, R57, A56, N73, P70, P67

10 Epi#48

Q202, K204, P69, P67, G58
Q202, K204, P70, P67, G63
Q202, K72, P70, P67, G66

15 Epi#49

D125, D43, L45, V78, Q42, Q39, T37, K38
D125, D43, L45, V78, Q42, Q39, T37, K41

Epi#50

20 H98, Y96, W90, L22, S21
H98, Y96, W90, P24, S33

Epi#52

F0, A16, R102, W90, N25, Q95
25 F0, A16, R102, W90, N25, Q93

Betv1:

30

Epi#03

SAS: 270, Size 11.07: L24, K20, H76, I23, Y81
SAS: 204, Size 11.96: L24, K20, A16, I23, Y81

35 Epi#05

SAS: 298, Size 14.01: G110, A106, A16, P14, G111, T10
SAS: 242, Size 14.01: G110, A106, A16, P14, G111, T107

Epi#08

40 SAS: 464, Size 11.12: K123, E127, G1, H121, F3
SAS: 455, Size 12.95: K129, E127, G1, H121, F3
SAS: 438, Size 13.31: K123, D125, G1, H121, F3
SAS: 428, Size 11.12: K123, E127, V2, H121, F3
SAS: 425, Size 11.65: K123, E127, G124, H121, F3

45

Epi#09

SAS: 466, Size 20.55: D109, A106, V105, K80, A16, T77
SAS: 444, Size 20.55: D109, G110, V105, K80, A16, T77
SAS: 427, Size 20.55: D109, G111, V105, K80, A16, T77
50 SAS: 398, Size 19.17: T10, G110, V105, K80, A16, T77

SAS: 381, Size 19.17: T10, G111, V105, K80, A16, T77

Epi#10

SAS: 558, Size 15.18: D75, T77, N78, A106, F79, R17, K20

5 SAS: 549, Size 21.96: E6, T7, N4, F3, G1, K123

SAS: 517, Size 13.31: D75, T77, N78, A16, F79, R17, K20

SAS: 497, Size 15.13: D75, T77, N78, A16, F22, R17, K20

Epi#12

10 SAS: 335, Size 9.08: T7, Y5, E6, N4

SAS: 331, Size 11.28: R145, Y150, E148, L152

SAS: 326, Size 10.37: R70, Y83, E73, P50

SAS: 311, Size 10.32: I116, Y5, E6, N4

SAS: 308, Size 8.33: R145, Y150, E148, S149

15

Epi#18

SAS: 328, Size 24.67: S117, K103, F79, V105, A16, Y158, L24

Epi#22

20 SAS: 533, Size 9.96: D125, D93, K123, E127

SAS: 533, Size 9.96: D93, D125, K123, E127

SAS: 476, Size 11.40: D125, D93, K123, E96

SAS: 476, Size 11.40: D93, D125, K123, E96

SAS: 400, Size 17.99: D125, D93, P90, E87

25

Epi#23

SAS: 451, Size 22.02: K68, N43, E42, S57, F64, P63

SAS: 450, Size 22.02: K55, N43, E42, S57, F64, P63

SAS: 428, Size 22.02: K68, N43, E42, S57, L62, P63

30 SAS: 427, Size 22.02: K55, N43, E42, S57, L62, P63

SAS: 412, Size 18.85: K68, N43, E42, S40, F30, P35

Epi#24

SAS: 734, Size 18.92: E127, K123, E96, P90, S136, E131, K129

35 SAS: 729, Size 18.92: D93, K123, E96, P90, S136, E131, K129

SAS: 716, Size 19.57: E127, K123, E96, P90, S136, E131, K134

SAS: 711, Size 19.57: D93, K123, E96, P90, S136, E131, K134

SAS: 708, Size 20.49: D125, K123, E96, P90, S136, E131, K129

40 Epi#25

SAS: 467, Size 12.68: R70, K55, I44, E42, E45

SAS: 425, Size 12.68: R70, K54, I44, E42, E45

SAS: 420, Size 14.01: R70, K55, I44, D27, E42

45 Epi#27

SAS: 613, Size 14.25: D93, E127, A130, E131, K129

SAS: 595, Size 16.54: D93, E127, A130, E131, K134

SAS: 592, Size 16.70: D125, E127, A130, E131, K129

SAS: 574, Size 19.79: D125, E127, A130, E131, K134

50 SAS: 524, Size 18.78: D93, E127, A130, E131, K137

Epi#28

SAS: 869, Size 21.93: V33, Q36, F58, E60, L62, F64, P63, K65
SAS: 837, Size 21.83: V33, Q36, F58, E60, L62, F64, G61, K65
5 SAS: 808, Size 24.56: V33, Q36, F58, E60, L62, F64, P90, K65
SAS: 783, Size 21.83: V33, Q36, F58, E60, K65, F64, S57, K68
SAS: 782, Size 21.83: V33, Q36, F58, E60, L62, F64, S57, K65

Epi#29

10 SAS: 516, Size 9.52: G61, K65, L62, E60
SAS: 440, Size 8.70: G61, P63, L62, E60
SAS: 371, Size 6.78: G61, P59, L62, E60

Epi#32

15 SAS: 374, Size 17.88: F79, A16, A106, D109, V12
SAS: 354, Size 20.42: F22, A16, A106, D109, V12

Epi#33

SAS: 541, Size 18.79: K65, F64, P90, S136, A135, K134
20 SAS: 498, Size 9.15: Q36, F30, P35, S39, K32
SAS: 496, Size 11.27: Q36, F30, P35, S40, K32
SAS: 494, Size 12.19: Q36, F58, P35, S39, K32
SAS: 493, Size 18.79: K65, Y66, P90, S136, A135, K134

Epi#36

25 SAS: 447, Size 19.17: T77, A16, A106, V12, G110, T10
SAS: 430, Size 19.17: T77, A16, A106, V12, G111, T10
SAS: 392, Size 19.17: T77, A16, A106, V105, G110, T10
SAS: 391, Size 19.17: T77, A16, A106, V12, G110, T107
30 SAS: 375, Size 19.17: T77, A16, A106, V105, G111, T10

Epi#40

SAS: 246, Size 21.55: A106, A16, Y158, S155
SAS: 223, Size 13.25: A135, A130, Y5, T7
35 SAS: 196, Size 14.88: A135, A130, Y5, S117
SAS: 178, Size 10.62: A135, G140, T142, S136

Epi#44

SAS: 530, Size 19.04: L24, R17, D156, Y150, S149, V12, T10
40 SAS: 492, Size 19.04: I23, R17, D156, Y150, S149, V12, T10
SAS: 490, Size 17.39: L24, R17, D156, Y150, S149, V12, P14
SAS: 483, Size 23.09: L24, R17, D156, Y158, A16, A106, P108
SAS: 474, Size 20.83: L24, R17, D156, Y150, S149, V12, T107

Epi#45

45 SAS: 606, Size 21.41: K32, P35, F30, Y150, R145, V12
SAS: 546, Size 20.89: K32, P31, F30, Y150, R145, V12
SAS: 533, Size 15.19: K32, P35, F30, Y150, R145, G140
SAS: 533, Size 12.63: K32, P35, F30, Y150, R145, V33
50 SAS: 532, Size 19.60: K32, P35, F30, N28, D27, I44

Epi#47

SAS: 333, Size 21.03: R17, L24, N28, P31, P35

SAS: 300, Size 22.72: R17, L24, N28, P31, S39

5 SAS: 298, Size 21.80: R17, L24, N28, P31, S40

SAS: 269, Size 24.87: R17, L24, N28, P31, S57

Epi#48

SAS: 436, Size 14.26: S57, K65, P90, P63, G61

10 SAS: 414, Size 17.96: S39, K32, P35, P59, G61

SAS: 412, Size 17.96: S40, K32, P35, P59, G61

SAS: 389, Size 18.32: S57, K65, P63, P90, G92

SAS: 365, Size 21.15: S57, K65, P59, P35, V33

15 "SAS" is solvent accessible surface. "Size" is the total surface
area of the epitope in Å².

Derf2:

20

Epi#02

A98, K100, S101, P99, R128, R31

A98, K100, R128, P99, R31, V94

T91, N93, P95, P34, R31, R128

25 L61, N93, P95, P34, R31, R128

Epi#03

L40, K15, A39, I13, Y86

L40, K14, A39, I88, Y90

30

Epi#05

G32, A98, R31, P34, G20, T36, T91, Y90

G32, A98, R31, P34, G20, T36, T91, V94

G32, A98, R31, P34, G20, T36, T91, L37

35 G32, A98, R31, P34, G20, T36, T91, V18

Epi#06

A98, P99, D129, R31, K96, P95

G32, P99, D129, R128, R31, P95

40 A98, P99, D129, R31, K33, P95

A98, P99, D129, R31, K96, P34

A98, P99, D129, R128, K126, P26

Epi#07

45 T107, S57, D59, S101, R128, A98, P99, D129

T107, S57, D59, S101, R31, A98, P99, D129

Epi#08

K15, D87, V76, H74, F75

50 K14, D87, V76, H74, F75

242

K77, D87, V76, H74, F75

Epi#09

L61, D64, I68, H74, F75, T70, N71
5 N114, N46, D113, K48, N71, T70, T49
G83, N46, D113, K48, N71, T70, T49

Epi#10

L40, I13, D42, N44, V81, K48, N46, N114, G115
10 L40, I13, D42, N44, V81, K82, N46, N114, G115
L37, D19, G20, V18, V3, D4, K6, A120, T107, V105

Epi#11

F75, K51, I111, Q45, V116, D113
15 F75, K51, I111, Q45, V81, D113

Epi#12

Y90, E38

20 Epi#13

H30, R31, P95, A98, P99, S101, G60, L61

Epi#15

K96, P99, D129, I28, R128, A98
25 K96, P99, D129, I127, R128, A98
K96, P99, D129, I29, R128, A98
K55, P66, D64, I68, T70, G67

Epi#18

30 R31, R128, I28, G125, T123, H124, V105
R31, R128, I127, G125, T123, H124, V105

Epi#22

D1, M17, D4, V3, K6
35 D1, M17, D19, P34, K96
D1, M17, D4, V5, K6

Epi#23

K14, N11, E12, N44, Q85, P79
40 K14, N11, E12, N10, Q45, P79
K14, N11, E12, N44, Q84, P79
K14, N11, E12, L40, Q85, P79

Epi#24

45 D129, K100, E102, P99, R128, R31, K96
E62, G60, E102, P99, R128, R31, K96
D129, K126, E102, P99, R128, R31, K33
D129, K126, E102, P99, R31, P95, K96

50 Epi#25

R31, K96, I97, D59, E62
R128, R31, I97, D59, E102
R128, K126, I127, E102, N103

5 Epi#27

D64, E62, D59, K100
D59, E62, D64, K55
D87, E38, D19, K33
D19, E38, D87, K15

10 D19, E38, D87, K14
D19, E38, D87, K77

Epi#28

V16, D87, Q85, K14, E12, K15, Q2, D1
15 I13, D87, Q85, K14, E12, K15, Q2, D1
V3, D1, Q2, K15, E12, K14, Q85, D87
L40, D87, Q85, K14, E12, K15, Q2, D1
I88, D87, Q85, K14, E12, K15, Q2, D1
V76, D87, Q85, K14, E12, K15, Q2, D1
20 V18, D1, Q2, K15, E12, K14, Q85, D87

Epi#29

G32, N93, L61, E62
V94, N93, L61, E62

25

Epi#30

G60, I97, A98, H30, K96, P34, P95
I68, N71, H74, K77, P79, V81
G32, I97, A98, H30, K96, P95, P34

30

Epi#34

V105, P26, S24, G125, R128, S101, P99
W92, P34, T91, V94, R31, S101, P99
I28, P26, T123, G125, R128, S101, P99

35

Epi#37

A120, V16, L40, K14, N11
A39, V16, L40, K14, N11
Y90, A39, L40, K14, N11
40 Y86, A39, L40, K14, N11

Epi#39

A120, E38, T91, P34, G20, L37
A39, E38, T91, P34, G20, L37

45

Epi#40

G20, L37, A120, T123, K6, S24
A39, L37, A120, T123, K6, S24
G20, L37, A120, T107, K6, T123

50

Epi#41

P34, L37, V106, S57

Epi#42

5 P26, S24, G125, R128, R31
P99, S101, G125, R128, R31

Epi#44

V16, Q2, D19, P34, W92, Y90, A39, V18, T91
10 V16, Q2, D19, P34, W92, Y90, A39, V5, T123
V3, Q2, D19, P34, W92, Y90, A39, V18, T91

Epi#45

K77, H74, F75, N71, D69, G67
15 K77, H74, F75, N71, D69, V76
K77, H74, F75, N71, D69, V65

Epi#46

A98, R128, R31, P95, N93, G32
20 A98, R128, R31, P34, G20, Q2

Epi#48

Q2, D19, P34, P95, G32
H30, K96, P95, P34, G20
25

Epi#49

D87, D42, L40, Q85, Q84, C78, T47, Q45, K48
D87, D42, L40, Q85, Q84, C78, T47, Q45, K82

30 Epi#50

D19, W92, P34, T91
D19, W92, P34, P95
D19, W92, T91, T36

35 Epi#51

D129, H30, K33, R31, R128, K126, H124
R31, H30, D129, R128, K100, K126, H124
T123, H124, K126, R128, R31, K33, H30

40

Derp2:

Epi#03

L17, K89, A39, I13, Y86
45 L17, K89, A72, I88, Y90
L17, K89, A72, I52, Y90

Epi#04

K15, S1, Q2, K14, V16, L17
50 K15, S1, Q2, K14, A39, L17

245

K15, S1, Q2, K14, V40, I13

Epi#05

G60, A56, L61, P99, G32, R31, H30, I97

5 G60, A56, L61, P99, G32, R31, H30, I28

Epi#06

G60, A56, D64, S57, K55, P66

G83, N46, D114, T49, K48, P79

10 G60, N103, D59, S101, R31, P95

Epi#08

K55, D64, S57, V106, F35

K55, E62, S57, V106, F35

15

Epi#09

L61, G60, E102, R128, I28, K126, N103, T123, V105

L61, G60, E102, R128, I127, K100, N103, T123, V105

L61, G60, E102, R128, I127, H124, N103, T123, V105

20

Epi#10

SAS: 435, Size 24.47: D69, T91, N93, F35, G32, R31

SAS: 422, Size 20.74: E38, T91, N93, F35, G32, K96

25 Epi#11

K14, I13, Q85, V81, E42

K15, I13, Q85, V81, E42

K14, I13, Q85, V40, D87

30 Epi#12

Y86, E42

Y90, E53

Y90, E38

35 Epi#13

H30, A125, P26, T123, A122, P19, L37, P34, W92

H30, A125, P26, T123, A122, H124, S24, G23, G20

H30, A125, P26, T123, A122, P19, L17, G20, F35

40 Epi#15

K55, P66, D69, I68, K89, A72

K55, P66, D69, I68, K89, A39

K55, P66, D64, I54, K109, G115

K55, P66, D64, I54, K109, A9

45

Epi#18

R31, I29, A125, S101, E102, N103

R31, I29, A125, S101, E102, V104

R31, I29, A125, T123, A122, V105

50

Epi#22

D69, P66, D64, V65, K55
D64, P66, D69, T91, K89
D59, L61, D64, P66, W92
5 D59, L61, D64, V65, E62
D69, P66, D64, V65, E53

Epi#24

D64, K55, E62, P99, R31, P34, K96
10 E53, K55, E62, P99, R31, P95, K96
D64, K55, E62, P99, R31, A98, K96

Epi#25

R31, H30, I28, E102, N103
15 R128, K126, I127, E102, N103
R128, K126, I28, E102, V105

Epi#27

D64, E53, D69, K89
20 D69, E53, D64, K55
D59, E62, D64, K55

Epi#28

V40, D87, Q85, E42, Q84, G83, K82
25 G20, H22, Q2, L17, E38, L37, Q36, P34, K33
G20, H22, Q2, L17, E38, L37, F35, P34, K33

Epi#29

I97, K100, L61, E62
30 G60, N103, L61, E62
I127, N103, L61, E62

Epi#30

G60, N103, S101, H30, K96, I97, P95
35 G60, N103, A125, H30, K96, I97, P95
I28, I127, A125, H30, K96, I97, P95

Epi#33

Q36, F35, V106, S57, A56, K55
40 K33, F35, V106, S57, A56, K55

Epi#34

I28, P26, S24, G23, G20, T123, S57
I28, P26, S24, V3, G20, T123, T107
45 W92, P34, T91, V18, G20, T123, P26

Epi#37

P66, V63, L61, K100, N103
P95, A98, L61, K100, N103
50 P19, V18, L17, K89, D87

247

P19, V3, L17, K89, D87
T123, V104, L61, K100, N103

Epi#38

5 L61, G60, E102, A125, V105, N103, P99, S57
L61, G60, E62, A56, V105, N103, P99, S57

Epi#39

A125, E102, H124, T123, P26, G20, L17

10

Epi#40

G60, L61, A56, T107, K6, T123
A39, L17, G20, T123, P26, S24
G60, L61, A56, T107, K55, S57
15 G60, L61, A56, T123, K126, S101

Epi#41

P19, L17, V3, S1
P19, L17, V5, S24

20

Epi#44

V65, D64, P66, W92, Y90, A39, V18, P19
L61, D64, P66, W92, Y90, A39, V18, T91

25 Epi#45

R31, P34, F35, N93, V94
K96, P34, F35, N93, G32

Epi#47

30 I127, S101, R31, I97, A98, L61, N103, P99, P95
I28, S101, R31, I97, A98, L61, N103, P99, S57

Epi#48

H30, K96, P95, P99, G60
35 H30, K96, P34, P19, G20
H30, K96, P34, P19, V18
H30, K96, P34, P95, V94
H30, K96, P34, P19, V3
E38, K89, P70, P66, V65
40 H30, K96, P95, P34, G32
Q36, K89, P70, P66, V65

Epi#50

D69, Y90, W92, P66, P70
45 D69, Y90, W92, P34, P95
D69, Y90, W92, T91, P34
D69, Y90, W92, V94, P95
D69, Y90, W92, L37, P19

50 Epi#51

248

K126, H124, E102, R128, I28, R31, H30
T123, H124, K126, R128, I28, R31, H30
D4, H124, K126, R128, I28, R31, H30

5 Phlp2:

Epi#02

T87, K85, Q61, S38, R34, R67
T87, K85, Q61, P63, R34, V42

10

Epi#03

K10, A90, I88, Y86
K10, A18, I88, Y86

15 Epi#04

R34, S38, Q61, K85, T87, I88
R34, S38, Q61, K85, T87, A90

Epi#05

20 G47, A18, S12, T87, G89, T91, T5, V1
G73, A29, L69, T27, G50, T53, T45, V42
G11, A18, L20, T91, G89, A90, T87, I88

Epi#06

25 A93, P94, D79, R34, Q61, P59
A93, P94, D79, R34, Q61, P83
A93, P94, D80, R34, Q61, P59
A93, P94, D79, R34, Q61, P63

30 Epi#08

K10, E9, G11, A18, H16, F54
K46, E48, G47, A18, H16, F54
K10, E9, S12, A18, H16, F54

35 Epi#09

L69, T27, G73, N76, R67, V77, D79, R34, A43, T45, V42
L69, T27, A29, E30, R67, V77, D80, R34, A43, T45, V42

Epi#10

40 D55, A18, N13, S12, F54, G47, K46
T45, A18, N13, S56, F54, G47, K46

Epi#09

L60, S56, E57, D55, K15, N13, S12, G11
45 L60, S56, E57, D55, H16, F54, T45, T53
L60, S56, E57, D55, H16, F54, T45, G47

Epi#12

Y86, E84
50 Y23, E24

Epi#18

N76, R67, F78, V81, A93, Y92, T91, T5, P2, V1

5 Epi#19

D39, W41, S38, Q61, R34, G37

E40, W41, S38, Q61, R34, A43

Epi#22

10 D79, P94, D80, P83, K85

D79, P94, D80, P63, K85

Epi#23

K10, N13, E14, L60, Q61, P59

15 K10, N13, E14, L60, Q61, P83

K10, N13, E14, L60, Q61, P63

Epi#24

E58, K15, E57, P59, S56, E14, Q61

20 D55, K15, E57, P59, S56, E58, Q61

Epi#25

R34, R67, W41, D39, E40

25 Epi#26

S38, E40, W41, V42, E32, E30

S38, E40, W41, V42, A43, E32

Epi#27

30 E14, E57, E58, K15

D55, E14, E84, K85

Epi#28

G37, H36, Q61, K85, E84, L60, F54, A43, K46

35 G37, H36, Q61, K85, E84, L60, F54, S12, D55

G37, H36, Q61, K85, E84, L60, F54, S56, D55

G37, H36, Q61, K85, E84, L60, F54, A43, R67

G37, H36, Q61, K15, E57, L60, F54, A43, K46

G37, H36, Q61, K85, E84, L60, F54, S12, K15

40 G37, H36, Q61, K85, E84, L60, F54, S56, K15

G37, H36, Q61, K85, E84, L60, F54, A43, R34

G37, H36, Q61, K85, E84, L60, F54, A18, D55

Epi#29

45 G73, K72, L69, R67, E30

I88, N13, L60, F54, E57

G25, K72, L69, R67, E32

V77, K75, L69, R67, E30

G37, H36, L60, F54, E57

50 G37, Q61, L60, F54, E57

Epi#30
I88, N13, S12, H16, K15, P59, L60
I88, N13, S56, H16, K15, L60, P59
5 I88, N13, A18, H16, K15, P59, L60

Epi#33
K46, F54, V42, S56, K15
H16, F54, V42, S56, K15

10 Epi#34
V1, P2, T5, V4, P94, Y92, T87
V1, P2, T5, L20, G89, T91, T87
V81, P94, T5, V1, P2, Y92, T91

15 Epi#37
T27, A29, L69, K72, D26
A43, R67, L69, K75, N76

20 Epi#38
L20, G89, E9, A18, N13, P59, S56

Epi#40
G49, L20, G89, Y86, K85, T87
25 G49, L20, G89, T87, K10, S12
G49, L20, G89, T87, K10, T7

Epi#44
V77, R67, D79, P94, Y92, A93, V1, P2
30 L69, R67, D79, P94, Y92, A93, V1, T5

Epi#45
D79, P94, F78, N76, M74, L69
D80, P94, F78, R67, D79, V77
35 K3, P94, F78, N76, M74, G73

Epi#46
A43, R67, R34, P63, H36, Q61
V77, R67, R34, P63, H36, G37
40 L69, R67, R34, P63, G37, Q61

Epi#47
G37, E35, E40, A43, R34, L60, N13, P59, S56
V77, E32, E40, A43, R34, L60, N13, P59, S56
45 S38, G37, E40, A43, R34, L60, N13, P59, S56

Epi#48
E24, K3, P94, P2, V1
E84, D80, P94, P2, V1

Epi#50

D39, W41, A43, T45

D39, W41, V42, T45

5 Epi#51

D79, H36, E84, T87, K10, G11, H16

D39, H36, Q61, K85, P63, R34, W41

D79, H36, E40, D39, G37, R34, W41

Q61, H36, E84, T87, K10, G11, H16

10

Example 11

15 For this example a third-generation epitope sequences were determined for some additional enzymes and redetermined for all of the enzymes in example 1-3. New enzymes are AMG (AMG.pdb), BPN¹ (lsup.pdb), Esperase (structure see Appendix D), Natalase (structure modelling based on SP722), Amylase-AA560 (Structure
20 modelling based on SP722), Protease A, Alcalase, Protease B, ProteaseC, ProteaseD, ProteaseE, Properase and Relase based on their sequences and structures. The structures of Protease B, Properase, Relase, Protease A, Alcalase, ProteaseC, ProteaseD and ProteaseE can be found by "Homology modelling" (see above)
25 and computer modelling of the epitope patterns that had been assembled in our database (shown in Table 8). Furthermore, the epitope sequences were redetermined for Carezyme, Laccase, PD498, Savinase, Amylase SP722, and Cellulase, according to the method.

30 The protein surface is scanned for epitope patterns matching the given "consensus" sequence of about 6-12 residues. First, residues on the protein surface that match the first residue of the consensus sequence are identified. Within a specified distance from each of these, residues on the protein surface that match
35 the next residue of the consensus sequence are identified. This procedure is repeated for the remaining residues of the consensus sequence. The method is further described under the para-

graph "Methods" above and the program can be found in Appendixes.

The critical parameters used in this screening included:

- 5 i) a maximal distance between the alfa-carbon atoms of subsequent amino acids,
- ii) a minimal accessibility of the amino acid of 20\AA^2 ,
- iii) the largest maximal distance between the most
10 distinct amino acids should be less than 25\AA
- iv) the best epitope were taken,
- v) the homology with the epitope pattern of interest was 100%

15

In this way a number of potential epitopes are identified. The epitopes are sorted according to total surface accessible area, and certain entries removed:

- 20 1) Epitopes that contain the same protein surface residue more than once. These are artefacts generated by the described algorithm.
- 2) Epitopes which are "too big", i.e. where a distance between any two residues in the epitope exceeds a
25 given threshold.

The subtilisin sequences and positions mentioned in the following are not given in the BPN' numeration but in the subtilisins own numeration (see the alignment as described above in Tables
30 1A and 1B).

The epitope sequences found were:

AMG:

Epi#01

L104, P123, P107, R125, R122, N182, S184, Q172, T173

L104, P107, P123, R125, R122, N182, S184, Q172, S453

5 L104, P107, P123, R125, R122, N182, S184, Q172, T452

Epi#02

L234, R241, S240, F237, T173, Y175, R122, R125

L234, R241, S240, F237, T173, Y169, R125, R122

10 L234, R241, S240, F237, T173, Y175, R125, R54

Epi#03

L291, K404, I288, Y289

L66, K61, H254, I253, Y329

15

Epi#04

R122, Y175, S184, Q172, Y169, A454, I455

R122, Y175, S184, Q172, Y169, N171, A451

R125, Y175, S184, Q172, Y169, T452, A451

20

Epi#06

G31, A24, D25, S30, A27, P41

G146, N145, D144, T148, S149, P467

A471, N145, D144, T148, S149, P467

25

Epi#07

G294, T290, S405, D293, S287, R286, P307, D283

G294, T290, S287, D293, S296, R286, P307, D283

G207, T204, S200, D214, S209, R160, P157, D153

30 G294, T290, S405, D293, S287, R286, P307, D309

Epi#08

A27, D25, S30, V111, F49

A24, D25, S30, V111, F49

35

Epi#09

S149, T148, G146, N145, A471, R68, N69, T72, V470

S73, S76, T72, N69, R68, A471, N145, T148

40 Epi#10

D238, N182, N236, S240, F237, R241, K244

D238, T173, N182, S239, F237, R241, K244

Epi#11

45 F49, F109, I91, Q85, E113

Epi#12

Y363, E342

Y311, E308

50 Y175, E180

Epi#13

S119, W120, P123, A102, P94, S92, G90, L98

S119, W120, P123, A102, P94, S92, G96, G90

5

Epi#15

K244, P307, D283, I288, T290, G294

R160, P157, D153, I154, T462, G90

R286, P307, D283, I288, T290, G294

10

Epi#16

L410, P46, Y48, R413, S397, S394, A392, A393, N395

R160, P157, Y458, G456, S211, S209, A205, A201, D214

15 Epi#17

A201, S209, R160, S459

A205, S209, R160, S459

Epi#19

20 D44, N45, S411, Q409, R413, L410

D47, N45, S411, Q409, R413, L410

Epi#20

K61, P434, L66, L423, N427, D65, G70, D71

25

Epi#22

D357, S356, D349, V346, D345

D349, S356, D357, A359, D345

D357, S356, D349, L348, D345

30

Epi#23

K404, N292, E299, S298, L295, A300

K404, N292, E299, S296, L295, A300

35 Epi#24

D336, K337, E259, P258, S431, L332, K378

D336, K337, E259, P258, S431, R429, K378

D336, K337, A261, P258, S436, E259, Q338

40 Epi#25

R125, R122, W120, E180, N182

R241, K244, E308, N313

Epi#26

45 W212, S200, E198, W437, V197, G438, E259

W212, S200, E198, W437, V197, A201, D214

Epi#27

D283, E280, D349, K352

50 D403, E408, D406, K404

D349, E280, D283, K244
D349, E280, D283, K279

Epi#28

5 L332, D336, Q338, K337, E259, C262, P272, D345
V374, D336, Q338, K337, E259, C262, P272, D345
G339, D336, Q338, K337, E259, C262, P272, D345

Epi#29

10 L295, G294, L291, R286, E299
I288, K404, L291, R286, E299
L348, K352, L354, F380, E299

Epi#33

15 K352, Y355, V374, S371, S365, K337
K352, Y355, V374, S365, S340, K337

Epi#34

V463, W466, S468, V470, P467, T464, T462
20 I469, W466, S468, V470, P467, T464, T462
I154, W466, S468, V470, P467, T464, T462
V463, W466, S468, V470, P467, S465, T464

Epi#37

25 T362, A359, L348, K352, D357
T360, V346, L348, K352, D357
T362, A359, L348, K352, D349

Epi#38

30 G438, E259, A435, R68, L66, N69, P434, S431

Epi#39

A353, E299, R286, P307, G243, L234
A300, E299, R286, P307, G243, L234

35

Epi#40

A205, L143, G146, Y147, P467, T464
G146, L143, A205, T204, A201, S209
A451, A450, T448, P446, S444

40

Epi#41

P467, Y147, L143, V206, S149

Epi#42

45 L66, P434, S431, N430, R429, R428
L104, P123, S95, G101, P94, R122, R125
L104, P107, S95, G96, P123, R125, Q172

Epi#44

50 L143, Q140, D144, W141, Y147, S468, V470, T72

256

V206, Q140, D144, W141, Y147, S468, V470, P467
S211, Q216, D214, P218, Y223, A451, A450, T448
S211, Q216, D214, P218, Y223, A450, G447, T448

5 Epi#45

R413, P46, F49, Y50, N110, D112, G31
R413, P41, F49, Y50, N110, D33, G31
D44, P46, F49, Y50, N110, D112, G31

10 Epi#46

Y175, R125, R122, P123, G174, Q172
Y169, R125, R122, P123, G174, Q172
V432, R429, R428, P434, N69, G70
Y175, R125, R122, P94, N93, G90

15 Y175, R122, R125, P123, N182, G121

Y175, R125, R122, P94, G101, A102
Y175, R125, R122, P94, G118, A115
Y175, R125, R122, P94, G101, G96
Y175, R122, R125, P123, N182, G183

20

Epi#48

S211, D214, P218, P446, G447
E259, K337, P258, P434, V432
S215, D214, P218, P446, G447
25 S209, D214, P218, P446, V445
E259, K337, P258, P434, V433

Epi#50

R122, Y175, W120, T117, S119
30 R125, Y175, W120, S119, T117

Epi#51

T390, H391, E408, Q409, R413, S411, W317
T390, H391, E408, S405, I288, K404, W317
35 D406, H391, E408, Q409, R413, S411, W317
T390, H391, E408, D406, K404, Q409, W317

Epi#52

W437, A260, T266, R273, W228, D264, Q225

40

BPN':

Epi#02

45 T255, K256, S260, F261, P194, Y262, R186, V203
L257, K256, S260, F261, P194, Y262, R186, V203
T253, K256, S260, F261, P194, Y262, R186, V203

Epi#03

50 K141, A137, I108, Y104

257

K136, A137, I108, Y104
K136, A134, I108, Y104

Epi#04

- 5 K265, Y262, S188, Q185, R186, N184, L257
K265, Y262, S188, Q185, Y263, R186, L257
K265, Y262, S188, Q185, R186, N184, G258
K265, Y262, S188, Q185, Y263, R186, G258

10 Epi#05

G80, A1, N77, P40, G211, S38, S37, V44
G80, A1, N77, P40, G211, S38, S37, L42
G127, A152, N155, T164, G160, S158, S188, Y262

15 Epi#06

G211, N212, D36, S37, K43, P40
G80, N212, D36, S38, K43, P40
G211, N212, D36, S38, K43, P86

20 Epi#08

K256, D259, S260, F261
K43, D36, S38, V44, F58

Epi#09

- 25 S105, S132, A133, A137, D140, K141, A144, S145, N118
S248, T244, A144, S145, D120, K27, N118, A116, N117

Epi#10

- E54, T55, N57, S37, F58, G46, K43
30 T55, A48, N57, S37, F58, G46, K43
E54, T55, N57, S49, F58, G46, K43

Epi#11

K136, I108, Q103, V51, D98

35

Epi#12

Y171, E195

Epi#13

- 40 S101, W106, P52, T55, A48, P56, S49, G47, F58
S105, W106, P52, T55, A48, P56, S49, G47, W113

Epi#15

- N25, P239, D120, I115, K141, A144
45 N240, P239, D120, I115, K141, A144

Epi#16

Q271, P14, Y21, G20, Q19, S18, A15, A272, N252
Q59, P210, Y214, G211, S38, D36, D61, A99, D98

50

258

Epi#17

A187, S188, R186, S183

A187, S188, R186, S182

5 Epi#18

N184, R186, S188, G157, S158, T159, S161

N184, R186, S188, G157, S158, T159, S162

N184, R186, S188, G157, S158, E156, N155

N184, R186, S188, G157, S158, E156, F189

10

Epi19

E156, N155, S188, Q185, R186, L257

E156, N155, S188, Q185, R186, G258

E156, N155, S188, Q185, R186, A187

15

Epi#22

D197, S260, D259, L257, K256

D197, S260, D259, Y263, K256

20 Epi#23

N155, E156, S188, Q185, A187

Epi#24

E156, G166, E195, P194, S260, L257, K256

25 D259, G264, E195, P194, S260, L257, K256

D197, K170, E195, P194, S260, L257, K256

Epi#25

K141, I115, D120, N25

30 K141, I115, D120, N118

K141, I115, E112, N118

Epi#26

W113, S49, W106, P52, E54, D98

35 W113, S49, W106, P52, E54, D60

W113, S49, W106, V51, E54, D98

Epi#28

A99, D61, Q59, F58, E54, L96, Q103, G102, D98

40 A99, D98, Q59, F58, E54, L96, Q103, G100, D61

A99, D61, Q59, F58, E54, L96, Q103, S101, D98

Epi#29

G102, Q103, L96, E54

45 G100, Q103, L96, E54

Epi#30

I79, N76, S87, H17, S18, P14, V4

I79, N76, S87, H17, Q19, P14, V4

50

Epi#31

L257, Q185, N184, R186, F189, V203, I205, D181

L267, Q10, N184, R186, F189, V203, I205, D181

5 Epi#33

K213, Y214, P210, S38, S37, K43

Q59, F58, V44, S38, S37, K43

Epi#34

10 W106, P52, M50, G47, P56, T55, S53

W106, P52, S49, G47, P56, T55, S53

I115, W113, M50, V51, P52, T55, S53

I108, W106, S105, V51, P52, T55, S53

15 Epi#35

A99, L96, S49, M50, I108

A99, L96, S49, M50, I107

Epi#36

20 A137, A134, A133, G131, Y104, S105, Q103, V51, A48, W113

A134, A137, A133, G131, Y104, S101, Q103, V51, A48, W113

Epi#37

Y262, R186, L257, K256, D259

25 Y263, R186, L257, K256, N252

Epi#39

E156, T164, P129, G127, L126

E156, T164, P129, G128, L126

30 E156, T164, P129, G154, L126

E156, T164, P129, G166, L126

Epi#40

R247, L250, A272, T255, K256, S260

35 R186, L257, G258, Y263, K256, S260

G264, L257, G258, T255, K256, S260

Epi#41

P194, Y262, L257, S260

40 P194, Y263, L257, S260

Epi#42

P194, S260, G258, R186, Q185

45 Epi#44

S182, Q185, D181, Y6, S9, V4, P14

S183, Q185, D181, Y6, S3, V4, P5

S248, R247, D197, P194, Y262, S260, G258, T255

S53, P52, W106, Y104, S105, V51, T55

Epi#45

K170, P194, F261, Y262, R186, D181, V203
D197, P194, F261, Y262, R186, D181, V203

5 Epi#46

S162, S158, E156, N155, A187, Q185, N184, R186, S188
S188, S158, E156, N155, A187, Q185, N184, R186, S183
S158, S188, E156, N155, A187, Q185, N184, R186, S182
S161, S158, E156, N155, A187, Q185, N184, R186, S183
10 G160, S158, E156, N155, A187, Q185, N184, R186, S188

Epi#48

S38, K43, P40, P210, G211
S37, K43, P86, P14, V4
15 S38, K43, P40, P210, G215

Epi#50

H238, W241, T242, P239
H238, W241, T244, T242
20 H238, W241, T242, T244

Epi#51

T242, H238, Q275, Q271, P14, S18, H17
Q245, H238, Q275, K237, P239, T242, W241
25 Q275, H238, Q245, T242, R247, T244, W241
Q245, H238, Q275, Q271, P14, Q19, H17

Carezyme Core:

30

Epi#01

P61, P165, K164, R158, N154, Y168, R153, S151
P137, P49, K44, K13, N32, Y54, Q36, T39
P61, P165, K164, R158, N154, S152, R153, S151

35

Epi#02

L115, N118, S117, R4, T6, Y147, R146, V129
L115, N118, S5, R4, T6, Y147, R146, V129

40 Epi#03

K44, A43, I38, Y54
K13, A43, I38, Y54

Epi#04

45 R153, S151, Q145, Y147, R146, I131
R153, S151, Q145, Y147, R146, G144
R153, S151, Q145, Y147, R146, L142

Epi#05

50 G3, A1, S183, T95, G101, A100, S96, G97

261

G3, A1, F184, T93, G101, T95, S96, G97
G97, A100, S96, T95, G101, T93, S183, G3

Epi#06

5 G140, P160, D161, R158, K164, P165
G50, P137, D133, R146, Q145, P143
A162, P165, D161, R158, K164, P160

Epi#07

10 G148, T6, S181, D178, R170, P165, D58
G128, T6, S181, D178, R170, P165, D58

Epi#08

K44, D42, S45, A43, F41

15

Epi#09

A191, E192, R196, A195, R200, N25, N202, N206
D161, R158, D157, R153, N176, S151, N154

20

Epi#10

D161, A57, N34, A162, F159, R158, K164
D2, A1, R185, S183, F184, G3, R4

Epi#11

25 F41, F29, I38, Q36, D58

Epi#12

Y168, E155
Y90, E91

30

Epi#13

A63, W62, P165, T60, A162, P160, L142, G149, Y147
A63, W62, P165, T60, A162, P160, L142, G128, Y147
A63, W169, P165, T60, A162, P160, L142, G144, Y147

35

Epi#15

P137, D133, I131, R146, G144
P137, D133, I131, R146, G148
P137, D133, I131, R146, G130
40 P137, D133, I131, R146, G128
P137, D133, I131, R146, G149

Epi#16

Q138, P137, Y54, R37, Q36, N34, A162, A57, D161
45 R170, P165, Y168, R153, S151, N176, D172, A63, D67
R170, P165, Y168, R153, S151, N176, D172, A63, D66

Epi#17

A1, S183, R4, S117
50 A100, S181, R4, S183

A1, S183, R4, S5

Epi#18

N118, R4, S181, ---, G3, ---, S117, L115, ---, A78, S80
5 N34, N32, R37, F35, ---, A33, Y54, S45, ---, ---, A43, V52

Epi#19

D157, N154, S151, Q145, R146, L142
D178, N176, S151, Q145, R146, G144

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Epi#22

D40, A43, D42, W18, K20
D40, A43, D42, A19, K20

15 Epi#23

R158, N154, E155, L142, Q145, P143
R153, N154, E155, S151, Q145, P143

Epi#24

20 D42, K44, E48, P137, F139, A33, Q36
D40, K44, E48, P137, F139, A33, Q36
D161, K164, A162, P160, R158, L142, Q145
D161, K164, E155, P143, R158, L142, Q145

25 Epi#25

R158, K164, W169, D172, N176
R4, H119, I77, E82, N81

Epi#26

30 W18, S15, E82, W85, P23, A19, D42
W18, S15, E82, W85, P23, G84, D203

Epi#28

I131, D133, Q138, L142, E155, K164, F159, P165, D161
35 I131, D133, Q138, L142, E155, K164, F159, P143, R158
I131, D133, Q138, L142, E155, K164, F159, P160, R158

Epi#29

I131, R146, L142, R158, E155
40 G144, Q145, L142, R158, E155

Epi#30

G79, N81, A78, H119, S117, I77, L115
G79, N81, A78, H119, S76, I77, L115

45

Epi#31

L142, R158, N154, R153, W169, F171, D172

Epi#33

50 Q36, F29, P27, S15, A19, K20

K44, F41, P27, S15, A19, K20

Epi#34

V129, P143, S151, G144, R146, Y147, T6
5 V129, P143, S151, G148, R146, Y147, T6
V129, P143, S151, G149, R146, Y147, T6

Epi#36

A83, A22, A19, S15, K13, V52, A43, W18

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Epi#37

Y147, R146, L142, R158, D161
Y147, R146, L142, R158, N154
Y147, R146, L142, R158, D157

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Epi#38

E155, R158, P160, G140, L142
E155, R158, P143, G144, L142

20 Epi#40

G79, L115, G113, T111, A74, T6
G79, L115, G113, T111, A74, S15
G79, L115, G113, T111, A74, S110
G116, L115, G113, T111, A74, T6
25 G79, L115, G113, T111, A74, S76

Epi#42

L142, P143, S151, G144, R146, Q145
L142, P143, S151, G148, R146, Q145
30 L142, P143, S151, G149, R146, Q145

Epi#44

L142, R158, D161, P165, W62, Y168, S152, G144, P143
I131, R146, D133, P137, Y54, A33, V52, P49
35 L142, R158, D161, P165, W62, Y168, S152, G149, P143

Epi#45

R185, P208, F207, N206, D203, V24
D67, P213, F68, N65, D66, V64
40 R185, P208, F207, N206, D204, G205

Epi#46

A195, R200, R201, P23, N202, G205
A191, R200, R201, P23, N202, G205
45 V24, R201, R200, P190, Q211, A209

Epi#47

A191, A195, E192, V194, R200, N202, R201, P23
A195, A191, E192, V194, R200, N25, R201, P23
50 A191, A195, R196, V194, R200, N202, R201, P23

Epi#48

E48, K44, P49, P137, V52

E48, K44, P49, P137, G50

5 E48, K44, P49, P137, G140

Epi#50

D172, Y168, W62, V64, P213

D42, W18, A43, T39

10 D67, W173, W62, V64, P213

D66, W173, W62, V64, P213

D42, W18, S45, P49

D172, W169, W62, V64, P213

15 Epi#51

R4, H119, D2, T95, P98, K175, W169

R4, H119, D2, R185, P208, Q186, W85

R4, H119, D2, T95, G97, K175, W173

20 Epi#52

W18, A22, R200, R201, W85, Q186

Esperase:

25

Epi#01

N24, P239, R237, K235, N243, S240, Q245, T242

N24, P239, K235, R27, N117, Y91, R43, S87

N24, P239, R237, K235, N243, Y241, Q245, S240

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Epi#02

T3, N76, L75, R43, S38, Y209, R213, V215

T3, N76, S87, R43, S38, Y209, R213, V215

T129, N166, Q161, R160, T156, Y192, R186, V203

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Epi#03

R186, Y192, S261, Q161, R160, N155, G127

R186, Y192, S261, Q161, R160, N155, G157

R186, Y192, S261, Q161, R160, N155, L126

40 R186, Y192, S261, Q161, R160, T156, G162

R186, Y192, S261, Q161, R160, N155, A187

Epi#05

G102, A105, S133, T134, G131, R170, T129, Y167

45 G102, A105, S133, T134, G131, R170, T129, G127

G211, A37, R43, P40, G80, T3, S78, I79

Epi#06

G211, N61, D97, R98, S53, P55

50 G102, N99, D97, R98, S53, P55

265

G100, N99, D97, R98, S53, P55

Epi#07

211, T210, D60, S38, R43, P86, D89

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Epi#08

A108, E136, S133, A105, F50

A108, E136, S132, A105, F50

A187, D181, S188, V203, F189

10

Epi#09

N212, G211, S38, H59, N61, N99, R98

S52, S53, R98, N99, N61, G211

15 Epi#10

T129, T156, N155, S188, F189, G157, R160

D181, N183, R186, S188, F189, G157, R160

T129, N166, N155, S188, F189, G157, R160

T129, T156, N155, S218, F189, G157, R160

20 D97, N99, N61, S57, F50, G102, R98

Epi#12

Y167, E136

Y192, E195

25 Y171, E136

Epi#13

S38, R43, P40, A37, H59, S57, P55, Y58

S38, R43, P40, A37, H59, S57, P55, F50

30 S38, R43, P40, A37, H59, S49, P55, Y58

Epi#15

N24, P86, D89, I44, R43, A45

N24, P86, D89, I44, R43, G46

35 N76, P86, D89, I44, R43, A45

N24, P86, D89, I44, R43, A37

Epi#16

Q161, P194, Y192, G157, R160, S188, D181, A187, N183

40 Q161, P194, Y192, R186, Q185, S188, D181, A187, N183

Q161, P194, Y192, G162, R160, S188, D181, A187, N155

Epi#17

A37, S38, R43, S87

45

Epi#18

N144, N140, R141, L137, S133, T134, E136, S132

N140, N144, R141, L137, S133, T134, A105, S103

N143, N144, R141, L137, S133, T134, E136, N140

50

266

Epi#19

I21, N18, Q15, Q275, R19, G20
I21, N18, Q15, Q275, R237, G20
E197, N265, S261, Q161, R160, G162
5 E197, N265, S261, Q161, R160, G157
I21, N18, Q15, Q275, R237, G25

Epi#23

R98, N61, E54, S53, F50, P55
10 R98, N61, E54, Y58, F50, P55
R98, N61, E54, S57, F50, P55
R98, N61, E54, S52, F50, A105

Epi#24

15 E195, G264, E197, P260, S261, P194, Q161
D89, G46, A48, P55, S52, F50, Q109
E197, G264, E195, P194, S261, L262, Q161

Epi#25

20 R98, H59, E54, N61
R98, H59, D60, N61
R43, H39, I44, D89, N24
R27, H120, I115, E112, N116

25 Epi#28

L104, Q109, I115, E112, W113, F50, S53, R98
A105, Q109, I115, E112, W113, F50, G102, R98
A108, Q109, I115, E112, W113, F50, S53, R98
V107, Q109, I115, E112, W113, F50, S53, R98

30

Epi#29

I147, N140, L137, R141, E136
G146, N140, L137, R141, E112
I115, N143, L137, R141, E136
35 G102, N99, L96, R98, E54

Epi#30

G211, N212, S38, H59, S57, I51, P55
G211, N61, S57, H59, S38, P40, L75
40 G211, N212, S38, H59, S49, I51, P55
G211, N212, S38, H59, P55, I51, L96

Epi#31

L257, Q185, N183, R186, F189, V203, D181
45 L262, Q185, N183, R186, F189, V203, D181

Epi#33

H59, Y58, P55, S52, S53, R98
Q109, F50, P55, S57, S53, R98
50 Q109, F50, P55, S49, S53, R98

Epi#34

I79, P40, S38, G211, R213, Y209, S216

I79, P40, S38, G211, R213, Y214, T210

5 I51, P55, S49, L96, R98, S53, S52

Epi#37

T134, A108, L137, R141, N144

Y256, A254, L257, R186, N183

10 A105, A108, L137, R141, N144

Epi#38

L257, G264, E195, L262, N265, P260, S259

L257, G264, E195, L262, N265, P260, S261

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Epi#39

E195, R170, P194, G264, L257

E195, R170, P194, G264, L262

20 Epi#40

R141, L137, A108, T134, A105, S133

R43, L42, A37, Y58, P55, S52

R186, L257, A254, Y256, P260, S259

R186, L262, G258, Y256, P260, S259

25 R186, L257, G184, Y256, P260, S259

R141, L137, A108, T134, A105, S103

R186, L262, G264, Y256, P260, S259

R186, L257, A254, Y256, P260, S261

R186, L262, G258, Y256, P260, S261

30 R186, L257, G264, Y256, P260, S261

Epi#41

P260, Y256, L257, S259

35 Epi#42

L75, P86, S87, N24, P239, R237, Q275

L75, P86, S87, N24, P239, R237, R19

Epi#44

40 S53, R98, D97, Y58, S57, A48, P55

S53, R98, D97, Y58, S38, G211, T210

Epi#45

R19, H17, F22, N24, D89, G25

45 R43, P86, F22, N24, D89, G25

R272, H269, F10, N183, D181, V203

R272, H269, F10, N183, D181, G184

R43, P86, F22, N24, D89, G46

50 Epi#46

268

R19, R237, P239, N24, G20

R19, R237, P239, N24, G25

Epi#47

5 G162, Y192, R160, N155, A187, Q185, N183, R186, S188
G157, Y192, R160, N155, A187, Q185, N183, R186, S188
S261, Y192, R160, N155, A187, Q182, N183, R186, S188
L262, Y192, R160, N155, A187, Q182, N183, R186, S188

10 Epi#48

S261, Q161, P194, P260, G258

S261, Q161, P194, P260, G264

Epi#50

15 D181, W6, V4, T3
D181, W6, V203, S188
D181, W6, V4, S9
D181, W6, T3, P5

20 Epi#51

R98, H64, T210, R213, P40, S38, H59
R98, H64, T210, R213, G211, S38, H59
R19, H17, Q15, Q275, R272, Q252, H269

25

Laccase:

Epi#02

A14, N15, S17, F21, P180, Y176, R266, V177
30 T22, N15, P18, F21, P180, Y176, R266, V177
A274, N275, A181, R175, P180, Y176, R266, V177
A24, N15, S17, F21, P180, Y176, R266, V177
T272, N275, A181, R175, P180, Y176, R266, V177

35 Epi#03

L184, K173, I186, Y256

Epi#04

R234, S211, Q261, K264, N267, G271
40 R234, S211, Q261, K264, R266, G268,
R259, S211, Q302, R234, N299, A301
R259, S211, Q236, R234, N299, A301

Epi#05

45 G372, A371, L369, P350, G81, S349, S351, V352
G372, A371, L369, P350, G81, S351, S349, Y347

Epi#06

G286, N289, D291, T293, S295, P292
50 G214, P252, D254, T293, S295, P298

A288, N289, D291, T293, S295, P292

Epi#07

G214, T294, D291, R283, V253, P252, D254

5 G30, T12, D53, R59, A497, P89, D51

G30, T10, D51, R59, A497, P55, D53

Epi#08

A371, E348, S349, A346, F335

10 A14, D53, G90, A92, H91, F93

A181, E183, G20, V16, F21

A181, E183, G20, A182, F21

Epi#09

15 N41, A100, N43, V6, D42, R37, N4, T8, L94

N41, A100, N43, V6, D42, R37, N4, T8, N47

L369, N366, E376, R379, N472, A471, V474

Epi#10

20 E183, A181, N275, T272, F273, G268, R266

D129, N41, N43, A100, F69, G72, R71

E183, A181, N275, A274, F273, G271, K264

Epi#11

25 F93, L486, I489, Q485, V481, E482

Epi#12

Y490, E488

Y375, E376

30

Epi#13

N366, P370, D367, I358, Q363, A471

N366, P370, D367, I358, Q363, G361

R379, P378, D326, I319, T321, G323

35 R379, P378, D326, I319, T321, G318

R379, P378, D326, I319, T321, A324

Epi#15

N366, P370, D367, I358, Q363, A471

40 N366, P370, D367, I358, Q363, G361

R379, P378, D326, I319, T321, G323

R379, P378, D326, I319, T321, G318

R379, P378, D326, I319, T321, A324

45 Epi#16

R175, P180, Y176, R266, Q164, N267, D166, A163, D205

R283, P292, Y256, G214, Q251, D254, A285, A288, N289

R283, P292, Y256, G214, Q251, D254, D291, A290, N289

50 Epi#17

A306, S413, R409, S414
A411, S413, R409, S414
A306, S410, R409, S414
A411, S414, R409, S410

5 Epi#19
E216, N250, Q251, Q191, R283, G286
E190, N250, Q251, Q191, R283, A288
E216, N250, Q251, Q191, R283, A290
10 E190, N250, Q251, Q191, R283, A285

Epi#22
D491, P494, D492, P495, E496
D492, P494, D491, L493, E496

15 Epi#23
R339, N460, E348, S349, L369, A371
R339, N460, E348, S351, L369, P370
R339, N460, E348, S351, L369, A365
20 R339, N460, E348, S351, L369, P350
R283, N188, E190, N250, Q191, P252

Epi#24
D475, G72, A476, P445, R379, A471, Q363
25 D53, G90, A497, P495, T498, P55, Q501
D53, G90, A497, P495, S499, L58, Q501

Epi#25
R37, K40, D129, N130
30 R37, K40, D129, N41

Epi#27
E142, E139, D138, K194,
E142, E139, D138, K193

35 Epi#28
L58, Q501, I500, E496, L493, P495, D492
G286, D254, Q191, K194, E190, K193, G192, D138
A288, D254, Q191, K193, E190, K194, G192, D138
40 G192, D248, Q191, K194, E139, L136, A135, D138
V253, D254, Q191, K193, E190, K194, G192, D138
A285, D254, Q191, K193, E190, K194, G192, D138

Epi#29
45 G390, Q332, L329, R330, E435
V374, N366, L369, E348
I500, P495, L493, E496
G344, Q332, L333, R330, E435

50 Epi#30

271

G412, N304, A306, H309, I312, P314, V419
I312, L311, A315, H309, P229, L136, P132

Epi#31

5 L329, Q332, N343, R330, F331, V386, D434
L333, Q332, N343, R330, F331, V386, D434
L58, Q501, N54, R59, F112, M459, F456, D205
L58, Q501, N54, R59, F112, M459, I454, D205

10. Epi#33

Q485, Y490, P494, S499, A497, R59
Q251, Y256, P292, S295, A296, R234
H153, F21, V16, S17, A182, K173
H153, F21, P18, S17, A182, K173

15

Epi#34

V431, P395, T432, G433, G412, T415, S414
V431, P388, T432, G412, G433, S414, T415
V419, P320, T321, G323, P322, Y416, S414
20 V431, P395, T432, G390, G433, S414, T415

Epi#35

A371, L369, A362, S360, M359, I358
G372, L369, A362, S360, M359, I358
25 A365, L369, A362, S360, M359, I358

Epi#36

A362, A471, A476, V474, G361, S360, Q357, P350, A371, A365
A290, A288, A285, V253, Y256, S295, A296, W257
30 A288, A285, A287, V253, Y256, S295, A296, W257

Epi#37

P132, A135, L136, K194, N250
A135, A134, L136, K194, D138
35 P298, A301, L303, R234, N299

Epi#38

L356, G81, B348, A371, V374, L369, N366, P370, S351
L356, G81, B348, A371, V374, L369, N366, P370, S349

40

Epi#39

A411, E435, T432, P395, G393, L392
A1, E142, L35, R37, P34, G30, L27
A389, E435, T432, P395, G394, L392

45

Epi#40

R330, L333, G390, T432, A411, S414
G393, L392, G394, T432, A411, S414
R330, L333, G390, T432, A411, T415

50

Epi#41

P370, L369, V352, S351

P350, L369, V352, S351

5 Epi#42

L392, P395, S428, G430, P388, R330, Q332

Epi#44

S360, Q363, D367, P370, Y347, A371, G372, T345

10 V253, Q191, D254, P292, W257, Y256, S295, A296, P298

S360, Q363, D367, P370, Y347, S349, V352, P350

V253, Q191, D254, P292, W257, Y256, S295, G214, P252

Epi#45

15 R409, P322, F418, Y416, N420, D313, V419

K423, P314, F418, Y416, N420, D313, V419

R175, P180, F21, Y176, R266, D166, G268

Epi#46

20 A296, R259, R234, P300, N299, A301

Y256, R259, R234, P300, N299, Q302

Epi#47

I212, S211, R234, L303, A301, N299, P300, P298

25 I212, S211, R234, V232, A301, N299, P300, P298

Epi#48

S158, Q160, P157, P155, V504

S499, Q501, P55, P155, V504

30 E488, Q485, P480, P479, V481

Epi#49

D367, L369, V352, P350, Q357, Q363, M359, N478

D367, L369, P370, P350, Q357, Q363, M359, N478

35

Epi#50

D291, Y256, W257, S295, P298

D254, Y256, W257, T293, S295

40 Epi#51

D307, H309, E228, T218, P229, T231, H230

R234, H215, E216, T231, P229, H230, H309

D248, H215, E216, T231, P229, H230, H309

45 Epi#52

F69, A100, T98, R71, W75, T73, Q70

F97, A100, T98, R71, W75, T73, Q70

50 Natalase:

Epi#01

P344, P382, R387, R33, N32, S28, R31, T36
P344, P382, R387, R33, N29, S28, R31, T36

5

Epi#02

A87, N21, Q18, R24, S28, R31, R33
A87, K89, S83, R24, S28, R31, R33

10 Epi#03

L307, K305, H402, I404, Y398
L307, K305, H401, I404, Y398
L307, K305, A304, I404, Y398

15 Epi#04

R167, S166, Q168, R172, N171, I173
R177, Y131, S128, Q125, R123, N124, I127

Epi#05

20 G178, A180, N124, P120, G190, S187, H234, L195
G178, A180, N124, P120, G190, R123, S187, Y192
G178, A180, N124, P120, G190, S187, H234, Y192

Epi#06

25 A87, N21, D25, R24, Q18, P14
G145, N146, D150, T147, R144, P142
G143, N146, D150, T147, R144, P142
G450, N451, D447, T455, K452, P453
A87, N21, D25, R22, Q18, P14
30 G454, N451, D447, T455, K452, P453
A378, P382, D447, T455, K452, P453

Epi#07

G145, T147, D150, S149, R213, V208, P205, D201

35

Epi#08

K305, D400, A304, H402, F399
K305, D400, A304, H401, F399

40 Epi#09

S79, S83, D25, R22, R24, H86, N90, S28, R31
N439, A460, N459, V444, K478, N417, T413, T414

Epi#10

45 E254, N249, R248, T245, F239, R212, R213
E254, N249, R248, T245, F239, R241, K275

Epi#11

F169, I173, Q170, D162
50 L195, I173, Q170, D162

Epi#12
Y192, E188
Y357, E354

5

Epi#13
H12, L13, P369, A375, P374, S372, P330, W11
H12, L13, P369, A375, P374, S372, P330, L334
H12, L13, P369, A375, P374, S372, P330, G331

10

Epi#15
N451, P453, D447, I448, T449, A378
N451, P453, D447, I448, K452, G450

15 Epi#16

Q313, P316, Y357, R353, Q395, D397, D400, A304, N308
Q355, P316, Y357, G356, R353, D397, D400, A304, D302

Epi#17

20 A87, S83, R24, S28
A87, S28, R24, S83

Epi#18

R33, N32, R31, S28, G92, N90

25

Epi#19
D16, N50, S48, Q49, R72, G69
D25, N21, Q80, Q18, R24, A87
E82, T77, Q18, Q80, R72, G69

30

Epi#22
D461, A460, W463, W433

Epi#23

35 K478, N417, E410, N439, Q438, A460
K478, N417, E410, N439, Q438, A441

Epi#24

E332, G331, E335, P330, S372, A375, K379
40 D381, K379, A375, P369, S372, P374, K377

Epi#25

R154, K138, W136, D162, N171
R213, R212, W217, E216, N249
45 R154, K138, W136, E134, N112
R241, K236, W183, D203, E206

Epi#26

W163, S166, E134, W136, V161, E117, E126
50 W163, S166, E134, W136, V161, E117, D130

275

W163, S166, E134, W136, V161, E117, D162

Epi#27

D203, E206, D201, K236

5 E117, E126, D130, K175

D201, E206, D203, K179

E126, E117, D162, K175

Epi#28

10 L195, D162, Q168, W163, E134, W136, Q165, S166, R167

I173, D162, Q170, W163, E134, W136, Q165, S166, R167

V161, D162, Q170, W163, E134, W136, Q165, S166, R167

Epi#29

15 G331, P330, L334, F337, E335

G178, K175, L114, R177, E117

Epi#30

G450, N451, H446, K478, I448, P453

20 G454, N451, H446, K478, I448, P453

Epi#31

Q168, N171, R172, W163, M196, I173, D162

Q170, N171, R172, W163, V161, I173, D162

25

Epi#33

K377, Y366, P369, S372, A375, K379

K377, Y366, P374, S372, A375, K379

30 Epi#34

W433, W463, T457, V444, G454, T455, P453

W433, W463, T457, V456, G454, T455, P453

Epi#37

35 Y156, R177, L114, K175, D130

T132, R177, L114, K175, N124

Epi#38

G429, E431, N469, P428, S472

40 G430, E431, N469, P428, S472

Epi#39

E10, H12, T370, P330, G331, L334

E10, L13, T370, P330, G331, L334

45

Epi#40

A378, A375, Y366, P369, S372

R177, L114, G178, Y156, K138, T110

A375, A378, Y366, P369, T370

50

Epi#41

P369, L13, V52, S48

Epi#42

5 P316, S281, G356, R353, Q355

P316, S281, G356, R353, Q395

Epi#44

V208, R213, W217, Y148, S149, G145, P142

10 S28, R33, D381, Y365, A378, A375, P369

L13, D16, P14, W11, Y362, A375, V373, T370

S333, D327, P330, W11, Y362, A375, V373, P369

Epi#45

15 D108, P142, F65, Y60, N146, D150, G145

D140, P142, F65, Y60, N146, D150, G145

Epi#46

Y392, R387, R33, P382, G450, G454

20 Y392, R387, R33, P382, Q388, G3

Epi#47

S83, S79, E82, I85, R24, A87, N90, R31, S28

A250, G252, E254, N249, R248, F256, N279, R241, S238

25

Epi#48

S372, H371, P374, P369, V373

Epi#49

30 D51, W11, L13, V52, P14, Q18, Q80, T77, N21

D51, W11, L13, V52, P14, Q18, Q80, T77, K74

Epi#50

D461, Y435, W433, W463, T457

35 D400, Y398, W433, W463, T457

D397, Y435, W433, W463, T457

Epi#51

T394, H396, D397, D400, K305, H402, H401

40 T455, H446, K478, T457, G442, Q438, W463

Epi#52

W136, A109, E134, R167, W163, N171, Q170

W136, A109, E134, R167, W163, N171, Q168

45

PD498:

Epi#02

50 T262, K258, S260, F266, T198, Y196, R168, V166

277

T262, K258, S260, F266, T264, Y196, R168, V166
T141, N139, Q171, F170, S167, Y196, R168, V166

Epi#03

5 L99, K51, A49, I53, Y56
L99, K51, A49, I53, Y43

Epi#04

R28, S331, Q333, K97, R50, I53
10 R28, S331, Q333, K97, R50, A49

Epi#05

G108, A106, N107, G110, S109, S111, I59
G110, A106, N107, G108, S109, S111, L112
15 G108, A106, N107, G110, S111, S117, Y121
G108, A106, N107, G110, S111, S109, G135
G110, A106, L68, P214, G217, S219, Y220
G108, A106, N107, G110, S111, S109, L134

20 Epi#06

G135, N163, D164, R168, S174, P176
G162, N165, D164, R168, S174, P176
A22, N274, D25, S2, S9, P6
G154, N152, D148, T142, K144, P176
25 A22, P21, D25, S2, S9, P6
G154, N152, D148, S145, K144, P176

Epi#07

29, T332, S331, D95, S240, R28, V26, P21, D25
30 G29, T332, S330, D95, S331, R28, V26, P21, D25

Epi#08

K258, D257, S260, F266
K190, D185, S192, V207, F193
35

Epi#09

N215, N44, R50, I53, K54, N64, N63, R61
N44, A49, R50, I53, K54, N63, N64, R61

40 Epi#10

D188, N187, R189, S260, F266, G263, K258
D185, N187, R189, S260, F266, G263, K258

Epi#12

45 Y268, E253

Epi#15

R50, P46, D82, I87, T83, G86
N215, P46, D82, I87, T83, G86

50

278

Epi#18

N216, N44, R50, I53, A49, P46, N215
N215, N44, R50, I53, A49, P46, N216

5 Epi#19

D95, T332, S240, Q241, R28, G29
D95, T332, S330, Q241, R28, G29

Epi#22

10 D185, S192, D164, Y196, K267
D105, S111, D113, T141, K144

Epi#24

D95, K51, A49, P46, R50, K97

15

Epi#25

R120, K153, W151, D148, N152
R189, K190, D188, N187
R189, K190, D185, N208

20

Epi#27

D201, E253, D257, K258
D257, E253, D201, K267

25 Epi#28

I259, D257, Q254, E253, K267, F266, S260, R189
I259, D257, Q254, E253, K267, F266, S260, K258

Epi#29

30 L68, G108, L134, F170, E137
G135, N163, L134, F170, E137

Epi#30

G110, N107, A106, H71, L68, L104, L112
35 G108, N107, A106, H71, L68, P214, V213
G110, N107, A106, H71, P214, L68, L104
G110, N107, A106, H71, L68, L104, L134

Epi#33

40 Q12, Y220, V207, S222, S192, R189
K190, F193, V207, S222, S192, R189
Q16, Y13, V207, S222, S192, R189

Epi#34

45 V26, W1, T27, G29, R28, S331, T332
W1, P21, T27, V26, R278, Y279, T255

Epi#35

G135, L134, S225, M221, I209
50 G110, L134, S225, M221, I209

279

G108, L134, S225, M221, I209
G162, L134, S225, M221, I209

Epi#37

5 A49, V52, L99, K54, N63
SAS: 309, Size 17.16: Y121, A127, L99, K54, N63
SAS: 307, Size 13.09: Y43, V52, L99, K54, N63

Epi#40

10 R189, G261, Y268, K258, S260
R189, G261, Y268, K258, T262

Epi#42

P3, S2, Q16, P21, R28, Q241

15

Epi#43

W199, Y196, G162, Q171, S140, L112, I115, T142

Epi#44

20 S145, D148, P176, W199, Y196, S167, G162, T169
S174, D201, P176, W199, Y196, S167, G197, T198

Epi#47

S330, S331, R28, V26, A22, Q16, N17, P21, S2
25 G242, S240, R28, V26, A22, Q16, N17, P21, S2
G29, S331, R28, V26, A22, Q16, N17, P21, S2

Epi#48

S2, D25, P21, P3, G86
30 S9, Q16, P21, P3, G86

Epi#50

R168, Y196, W199, T264, T198
D164, Y196, W199, T264, S260

35

Savinase:

Epi#01

40 L21, N18, P14, R19, K231, N232, S236, Q239, S234
L21, N18, P14, R19, K231, N232, S234, Q230, S24
L21, N18, P14, R19, K231, N232, S234, Q230, T22

Epi#02

45 T254, N255, A188, R164, S158, Y186, R180, V197
T249, N263, Q12, R10, P14, R19, R269
T249, N263, S9, R10, P14, R19, R269

Epi#03

50 K27, A86, I43, Y89

Epi#04

K229, S234, Q230, K231, R269, A266

K27, S24, Q230, K231, R269, A15

5 K231, S234, Q239, R241, N246, A248

Epi#05

G187, A188, N255, T254, G252, S250, T249, L251

G189, A188, N255, T254, G252, S250, T249, L261

10

Epi#06

G252, N179, D175, S182, S154, P127

A188, N255, D191, R164, S158, P127

A188, N255, D191, R164, S128, P127

15

Epi#08

A131, E134, S139, A106, F49

A166, E134, S139, A106, F49

20 Epi#09

S103, T132, A131, E134, A166, R164, N167, S142, R143

Epi#10

D175, N177, N179, S182, F183, G155, R180

25 D175, N212, N153, S182, F183, G155, R180

Epi#11

F49, L94, I105, Q107, V102, E134

F49, K92, I105, Q107, V102, E134

30

Epi#12

Y161, E134

Y165, E134

35 Epi#13

S76, L73, P39, T207, A209, P204, S206, G205, Y208

S85, L73, P39, T207, A209, P204, S206, G205, Y203

Epi#16

40 R164, P127, Y161, G152, S158, N255, D191, A166, N167

R164, P129, Y161, G152, S158, N255, D191, A166, N138

Epi#17

A156, S158, R164, S128

45 A188, S158, R164, S126

Epi#18

N177, N179, R180, S182, G155, S154, A156, S158

N177, N178, R180, S182, G155, S154, N153, F183

50

- Epi#19
D175, N179, S182, Q185, R180, L256
D175, N179, S182, Q185, R180, L251
I240, W235, S234, Q239, R241, K245
5 D175, N179, S182, Q185, R180, G252
- Epi#23
R143, N114, E110, S139, Q135, A131
R143, N115, E110, N138, Q135, A131
10
- Epi#24
D58, G59, E53, P51, F49, P54, Q57
D58, G59, E53, P51, S48, P54, Q57
D58, G59, E53, P54, S55, F49, Q107
15
- Epi#25
R19, R269, E265, N18
R269, R19, E265, N18
- 20 Epi#28
V102, Q107, F49, E53, K92, Q57, G46, R44
A47, Q107, F49, E53, K92, Q57, G46, R44
V50, Q107, F49, E53, K92, Q57, G46, R44
- 25 Epi#29
I77, N74, L41, R44, E87
V4, N74, L41, R44, E87
G20, N18, L21, R19, E265
- 30 Epi#30
G59, N60, S97, H62, L94, P51, P54
G98, N60, S97, H62, L94, P51, P54
- Epi#31
35 L256, R180, N178, R10, W6, V197, D175
L251, R180, N178, R10, W6, V197, D175
- Epi#33
Q107, F49, P51, S48, S55, K92
40 Q107, F49, P54, S55, A47, K92
- Epi#34
V102, P129, S128, G125, R164, Y161, P127
V102, P129, S126, G125, R164, S158, P127
45
- Epi#37
T254, A188, L256, R180, N177
T254, A188, L256, R180, N179
- 50 Epi#38

L94, G59, E53, A96, N60, P204, S206
L94, G59, E53, A96, N60, P204, S36

Epi#39

5 A131, E134, L133, T132, P129, G125, L124
A166, E134, L133, T132, P129, G125, L124

Epi#40

R44, L41, G78, T207, P39, T37
10 R19, L21, G20, T22, K231, S234
R180, L256, G252, T254, A188, S158

Epi#41

P127, Y161, L133, V102, S99
15 P127, Y161, L133, V102, S103
P127, Y161, L133, V102, S101
P127, Y161, L133, V102, S126

Epi#42

20 L73, P84, S85, N74, H17, P14, R19, R269
L80, P5, S3, N74, H17, P14, R19, R269
L21, P84, S85, N74, H17, P14, R19, R269

Epi#43

25 105, W111, A47, G46, Q57, S36, L41, I43, T37

Epi#44

S126, R164, P127, Y161, S158, A188, T254
S128, R164, P129, Y161, S158, A188, T254

30

Epi#46

A15, R269, R19, P14, N18, G20
A266, R269, R19, P14, N18, A15

35 Epi#48

S55, Q57, P54, P51, G52
E53, Q57, P54, P51, G52

Epi#50

40 R10, W6, S3, S76
R241, W235, S234, P233
R10, W6, V4, S9

Epi#51

45 Q239, H243, T247, R269, R19, K231, W235
R19, H17, E265, R269, K231, S234, W235

Epi#52

A15, S9, R10, W6, N198, Q176

A15, S9, R10, W6, N198, Q200

Amylase SP722:

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Epi#02

T419, N423, P422, F396, T5, Y398, R393, R37

T419, N418, P422, F396, T5, Y398, R393, R37

10 **Epi#03**

L313, K311, H408, I410, Y404

L313, K311, H407, I410, Y404

Epi#04

15 R171, S170, Q172, R176, N175, I177

R181, Y135, S132, Q129, R127, N128, I131

Epi#05

G184, A186, N128, P124, G196, S193, H240, L201

20 G184, A186, N128, P124, G196, R127, S193, Y198

Epi#06

G147, N150, D154, T151, R148, P146

G149, N150, D154, T151, R148, P146

25

Epi#07

G149, T151, D154, S153, R219, V214, P211, D207

Epi#08

30 K311, D406, A310, H407, F405

K311, D308, A310, H408, F405

Epi#09

T461, R485, K484, N423, T419, N418

35 R485, K484, N423, T420, T419

Epi#10

E260, N255, R254, T251, F245, R218, R219

T419, N423, N395, T5, F396, R393, R37

40 E260, T257, N255, T251, F245, R218, R219

Epi#11

F173, I177, Q174, D166

L201, I177, Q174, D166

45

Epi#12

Y363, E360

Y398, E360

Y198, E194

50

Epi#13

H16, L17, P375, A381, P380, S378, P336, W15
H16, L17, P375, A381, P380, S378, P336, G337
H16, L17, P375, A381, P380, S378, P336, L340

5

Epi#15

N457, P459, D453, I454, K458, G456
K458, P459, D453, I454, T455, A384
N457, P459, D453, I454, K458, G460

10

Epi#16

Q319, P322, Y363, R359, Q401, D403, D406, A310, N314
Q319, P322, Y363, G362, R359, D403, D406, A310, N314
Q319, P322, Y363, R359, R415, D403, D406, A310, N314

15

Epi#17

A91, S32, R28, S87
A91, S87, R82, S83

20

Epi#18

R485, V450, G448, T463, T461, H452, V462
N126, N128, R127, G196, Y198, S193, N195, N125
N25, R26, R28, S87, I89, A91, H90, N94

25

Epi#19

D20, N54, S52, Q53, R76, G73
D20, N19, Q22, Q84, R76, G73
D29, N25, Q22, Q84, R28, A91

30

Epi#20

K385, P350, L355, L313, K311, D308, G305, D432

Epi#22

D183, A186, D209, W189, K242
35 D183, A186, D209, W189, E190
D183, A186, D209, P211, E212
D209, A186, D183, Y160, W159
D183, A186, D209, W187, W189

40

Epi#23

R415, N418, E416, N445, Q444, A466
K446, N445, E416, Y441, Q444, A466

Epi#24

45 D387, K385, A381, P375, S378, P380, K383
E341, G337, E338, P336, S378, A381, K385
D333, G337, E341, P336, S378, A381, K385

Epi#25

50 R485, H452, I454, E391, N36

285

R485, K484, I454, E391, N395

Epi#26

W167, S170, E138, W140, V117, G182, D183
5 W167, S170, E138, W140, V165, E121, D134
W167, S170, E138, W140, V165, E121, E130

Epi#27

E212, E216, D154, K156
10 E216, E212, D209, K242

Epi#28

L201, D166, Q172, W167, E138, W140, Q169, S170, R171
L201, D166, Q169, W140, E138, W167, F173, S170, R171
15 L201, D166, Q174, W167, E138, W140, Q169, S170, R171

Epi#29

V214, N215, L217, R219, E222
G96, H90, L228, R82, E86
20 V214, R219, L217, R218, E212

Epi#30

G456, N457, H452, K484, I454, P459
G362, M323, S287, H324, K320, P322, V318
25 G362, M323, S287, H321, K320, P322, V318
G460, N457, H452, K484, I454, P459

Epi#31

L217, R219, N215, R218, F245, V214, D248
30 L217, R219, N215, R218, F245, M208, D209

Epi#33

K383, Y372, P375, S378, A381, K385
K383, Y372, P380, S378, A381, K38
35

Epi#34

W439, W469, T463, V450, R485, T461, P459
W439, W469, T463, V462, R485, T461, P459

40 Epi#37

T251, R218, L217, R219, N215
P211, V214, L217, R219, N215
A256, R218, L217, R219, N215

45 Epi#38

G435, E437, N475, P434, S478
G436, E437, N475, P434, S478

Epi#39

50 E338, H16, T376, P336, G337, L340

286

E14, H16, T376, P336, G337, L340

Epi#40

A384, A381, Y372, P375, S378

5 A384, A381, Y372, P375, T376

Epi#41

P375, L17, V56, S52

10 Epi#42

S378, P380, Y372, A381, A384, P375

S378, P375, Y372, A381, A384, P388

S378, P375, Y372, A381, A384, T455

15 Epi#45

K72, P146, F69, Y64, R148, D154, G149

K311, H408, F405, N409, D432, G304

D406, H408, F405, N409, D432, G304

20 Epi#46

Y398, R393, R37, P388, Q394, G7

Y398, R359, R393, P388, G456, G460

Y398, R393, R37, P388, Q394, G38

25 Epi#47

A256, G258, E260, N255, R254, F262, N285, R247, S244

S193, Y198, E194, N125, R127, Q129, N123, R176, P124

Epi#48

30 S378, H377, P380, P375, V379

H16, H377, P375, P380, V379

Epi#49

D55, W15, L17, P18, Q22, Q84, T81, N25

35 D55, W15, L17, P18, Q22, Q84, T81, K78

Epi#50

D467, Y441, W439, W469, T463

D406, Y404, W439, W469, T463

40 D183, Y160, W159, W140, T114

D403, Y441, W439, W469, T463

Epi#51

D406, H408, D308, K311, L313, Q319, H321

45

Epi#52

W140, A113, E138, R171, W167, N175, Q174

W140, A113, E138, R171, W167, D166, Q172

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Amylase AA560:

Epi#01

L390, P388, P350, K383, K385, N457, S478, R458, T461
5 L390, P388, P350, K383, K385, N457, S478, R458, T452
L390, P388, P350, K383, K385, N457, S478, R458, T455

Epi#02

L390, K395, Q394, R393, T5, Y398, R359, R400
10 L173, K172, S170, T136, Y135, R118, R181
L173, R171, S170, T136, Y135, R118, R181
L390, K395, Q394, R393, T5, Y398, R400, R415

Epi#03

15 K438, H407, I410, Y404

Epi#04

K172, S170, Q169, R171, N174, L173
R171, S170, Q169, K172, N175, I177

20

Epi#05

G456, A459, R458, T461, G460, T452, T463, V450
G456, A459, R458, T452, G460, T461, T463, G448

25

Epi#06

A51, N54, D20, R76, Q71, P146
G73, A51, D55, S52, K72, P146

Epi#07

30 G456, T455, S384, D387, R393, P388, D453

Epi#08

K259, S255, V222, H252, F245
K259, G258, A256, H252, F245

35

Epi#09

N128, V131, R176, D166, K172, N175, N174, R171

Epi#10

40 467, N445, R444, F441, R415, R400
D467, A466, R444, F441, R415, R400

Epi#11

F69, K72, I75, Q53, V56, D55

45

Epi#12

Y16, E337
Y363, E360
Y198, E194

50

Epi#15

K385, P388, D453, I454, R458, A459

K385, P388, D387, I454, T452, A459

K385, P388, D387, I454, R458, G456

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Epi#17

A87, S29, R28, S32

A91, S29, R28, S32

10 Epi#18

N445, R444, A466, T463, T461, N471, N437

N445, R444, A466, T463, T461, T452, V450

Epi#19

15 166, W167, S170, Q169, R171, K172

E138, W167, S170, Q169, R171, K172

E134, T136, S170, Q169, R171, K172

Epi#22

20 D209, P211, D207, Y160, D183

Epi#23

R400, N418, E416, N445, Q449, A466

R82, N83, E68, N70, F69, P146

25

Epi#24

E134, G133, E130, P124, R176, L173, K172

E134, K179, E130, P124, R176, L173, K172

30 Epi#25

R444, K446, W469, D467, N445

R171, K172, W167, D166, N175

R171, K172, W167, D166, N174

35 Epi#26

W167, S170, E138, W140, V165, E121, E130

W167, S170, E138, W140, V165, E121, E134

W167, S170, E138, W140, V165, E121, D166

40 Epi#27

E130, E121, D166, K172

D36, E391, D387, K385

E134, E121, D166, K172

45 Epi#28

L201, D166, Q169, W140, E138, K172, S170, R171

L173, D166, Q169, K172, E138, W167, S170, R171

Epi#29

50 V131, R176, L173, R171, E138

289

I177, N175, L173, R171, E138

I177, N174, L173, R171, E138

Epi#30

- 5 I39, N33, S29, H23, P18, L17, P375
G38, N33, S29, H23, L17, P375, P380
G362, M323, S287, H321, Q319, P322, V318
G417, N423, A420, H421, K395, L390, P388
G21, N25, S29, H23, P18, L17, P375
10 G399, N418, A420, H421, K395, L390, P388

Epi#31

L173, R171, N174, R176, W167, M202, I177, D166
L173, R171, N174, R176, W167, V165, I177, D166

15

Epi#33

K108, Y58, V56, S52, A51, K72

Epi#34

- 20 W439, W469, T463, V450, G460, T452, T461
W15, P18, T376, G378, P375, Y372, S384
W469, W439, S473, G460, R458, T461, T463

Epi#37

- 25 P124, R176, L173, K172, N175
P124, R176, L173, R171, N174

Epi#40

- R400, G399, Y396, P422, T419
30 R400, G417, Y396, P422, T419

Epi#41

P375, Y16, L17, V56, S52
P18, Y16, L17, V56, S52

35

Epi#42

P350, S478, G433, H408, R310, Q311
P322, S287, N285, H324, R320, Q319
P322, S287, G362, H321, R320, Q319

40

Epi#44

L17, D20, P18, W15, Y368, A381, G378, T376
L340, D333, P336, W15, Y368, A381, G378, P375

45 Epi#45

K72, P146, F69, Y64, N150, D144, G147
D112, P146, F69, Y64, N150, D144, G149

Epi#46

- 50 Y398, R359, R393, P388, G456, A459

290

Y363, R359, R393, P388, Q394, G7
Y363, R359, R393, P388, Q394, G38

Epi#47

5 I75, E68, R76, N83, R82, Q84, N90, R28, S29
G133, E134, E130, V131, R176, L173, N174, R171, S170

Epi#48

S384, K383, P380, P375, G378
10 E337, H377, P380, P375, V379

Epi#50

R444, W469, W439, S473, T461
D183, Y160, W159, W140, T114

15

Epi#51

R320, H321, Q319, P322, H324, H286

Epi#52

20 W140, A113, E138, R171, W167, D166, Q169
W140, A113, E115, R118, W159, T114, Q169

Protease A:

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Epi#01

L21, N18, P14, R19, K237, N238, S242, Q245, S240
L21, N18, P14, R19, K237, N238, S240, Q236, S24

30

Epi#02

T255, N269, Q12, R10, P14, R19, R275
T255, N269, S9, R10, P14, R19, R275

Epi#03

35 K27, A88, I44, Y91

Epi#04

K235, S240, Q236, K237, R275, A15
K27, S24, Q236, K237, R275, A15
40 K237, S240, Q245, R247, N252, A254
R145, S141, Q137, Y171, N173, A172

Epi#06

G61, N62, D60, T38, Q59, P55
45 G211, P210, D60, T38, Q59, P55
A98, N62, D60, T38, Q59, P55
G100, N62, D60, T38, Q59, P55

Epi#08

50 A131, E136, S141, A108, F50

A172, E136, S141, A108, F50
A98, E54, G53, V51, F50

Epi#09

5 S162, S170, A172, N173, V244, H249, N252, S256, T260
S259, S256, T260, N261, L262, R186, N185, S188, N155
S162, S170, A172, N173, V244, H249, N248, N252, T255
S156, S162, N261, S259, L262, R186, N185, S188, N155

10 Epi#10

D181, N183, N185, S188, F189, G157, R186
D181, N218, N155, S156, F189, G157, R186

Epi#12

15 Y171, E136
Y91, E89

Epi#13

S78, L75, P40, T213, A215, P210, S212, G211, Y209
20 S87, L75, P40, T213, A215, P210, S212, G211, Y214

Epi#16

L262, P194, Y192, G195, S162, N261, D197, A172, N140
L262, P194, Y192, G157, S162, N261, D197, A172, N173
25 L262, P194, Y192, G161, S162, S170, D197, A172, N173

Epi#17

A138, S141, R145, S144
A108, S141, R145, S144

30

Epi#18

N185, N183, R186, L262, S259, T260, P194, N261
N185, N183, R186, L262, Y192, T260, P194, S162

35 Epi#19

I246, W241, S240, Q245, R247, K251
D181, N185, S188, Q191, R186, L262

Epi#23

40 R145, N116, E112, S141, Q137, A138
R145, N117, E112, S141, Q137, A108

Epi#24

E136, G133, A131, P129, S103, F50, Q109
45 E136, G132, A131, P129, S103, A108, Q137
D60, G61, E54, P52, F50, P55, Q59

Epi#25

R275, R19, E271, N18

50

Epi#28

G20, H17, Q12, E271, L21, Q236, S240, K237
A15, H17, Q12, E271, L21, Q236, S240, K237

5 Epi#29

V244, Q245, L148, R145, E112
V244, N173, L148, R145, E112

Epi#30

10 G61, N62, A98, H64, L96, P52, P55
G20, N18, A15, H17, S87, L75, P40
I79, N76, S87, H17, Q12, P14, V4
G100, N62, A98, H64, L96, P52, P55

15 Epi#31

L262, R186, N184, R10, W6, V203, D181
L257, R186, N184, R10, W6, V203, D181

Epi#33

20 Q109, F50, P52, S49, S56, K94
Q109, F50, P55, S56, A48, K94

Epi#34

W241, P239, S242, G146, R145, S141, S144
25 I165, P194, T260, G258, R186, S188, S156
V104, P129, S130, G127, G102, S101, S99
V244, W241, S242, G146, R145, S141, S144
I165, P194, S170, G127, P129, S130, S103

30 Epi#37

P14, A15, L21, R19, N18
T143, R145, L148, R247, N252
T143, V244, L148, R145, N116

35 Epi#38

L96, G97, E54, A98, N62, P210, S212
L96, G97, E54, A98, N62, P210, S37

Epi#39

40 A15, E271, H17, R19, P14, G20, L21
A254, E271, H17, R19, P14, G20, L21
A272, E271, H17, R19, P14, G20, L21

Epi#40

45 R186, L257, G258, T260, P194, S162
R186, L262, G161, Y192, P194, T260

Epi#41

P194, Y192, L262, S259
50 P194, Y192, L196, S162

Epi#42

L82, P5, S3, N76, H17, P14, R19, R275

L82, P5, S9, Q12, H17, P14, R19, R275

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Epi#43

W113, A48, G47, Q59, S37, L42, I44, T38

Epi#44

10 V244, R247, D197, P194, Y192, S162, G195, T260

V244, R247, D197, P194, Y192, S170, G195, T260

S56, Q59, D60, P210, Y214, S212, G211, T38

S56, Q59, D60, P210, Y209, S212, G211, T38

15 Epi#46

A15, R275, R19, P14, N18, G20

A272, R275, R19, P14, N18, G20

A272, R275, R19, P14, N18, A15

20 Epi#47

S130, A131, E136, N173, A172, N140, R145, S144

S105, A131, E136, N173, A172, N140, R145, S144

Epi#48

25 E54, Q59, P55, P52, G53

S56, Q59, P55, P52, G53

S49, Q59, P55, P52, G53

Epi#50

30 R10, W6, S3, S78

R10, W6, V4, S9

R10, W6, V203, S188

Epi#51

35 Q245, H249, T253, R275, K237, S240, W241

R19, H17, E271, R275, K237, S240, W241

R145, H120, K27, S24, K237, S240, W241

R145, H120, K235, K237, P239, S240, W241

40 Epi#52

A15, S9, R10, W6, N204, Q206

A15, S9, R10, W6, N204, Q182

45 **Alcalase:**

Epi#01

L10, P5, P9, K15, K12, N269, S251, R249, T253

L82, P5, P9, K15, K12, N269, S251, R249, T253

50

Epi#02

T115, N141, A144, R145, S242, R247, R249
A138, N141, A144, R145, S242, R247, R249

5 Epi#03

L196, K170, A129, I165, Y167
L196, K170, A194, I165, Y171

Epi#04

10 R145, Y143, S173, Q137, K136, T133, A134
K170, Y167, S132, Q137, K136, N141, A144

Epi#05

G53, A52, F50, G102, S105, S103, Y104
15 G53, A52, F50, G102, S101, S103, Y104

Epi#06

A24, N25, D120, R145, S242, P239
A144, N141, D140, R145, S242, P239

20

Epi#08

K265, E197, S260, A194, F261
A56, E54, G53, A52, F50

25 Epi#10

T162, N161, N163, A194, F261, G264, K265
E195, N161, N163, S158, F261, G258, K265

Epi#12

30 Y57, E54
Y262, E197

Epi#13

S38, A37, P40, T213, A215, H64, L217, G204, Y206
35 S38, A37, P40, T213, A215, H64, S98, G100, G61
S87, L75, P40, T213, A215, H64, L217, G204, Y6

Epi#16

L10, P9, Y6, G204, S182, N183, D181, A187, N185
40 Q2, P5, Y206, G204, S182, N183, D181, A203, N218
L10, P9, Y6, G204, S182, N183, D181, A187, N155

Epi#17

A144, S244, R247, S252
45 A272, S252, R249, S244
A144, S244, R249, S251
A254, S252, R249, S244

Epi#18

50 N141, R145, A144, Y143, S244, N248, S252

Epi#19

N248, S244, Q245, R249, A272

N240, S242, Q245, R249, A254

5 N240, S242, Q245, R249, L241

Epi#22

D76, L82, D14, A18, K15

D181, L10, D14, A18, K15

10

Epi#23

K27, N117, E112, N141, Q137, A134

K27, N117, E112, N141, Q137, A138

K27, N117, E112, S109, F50, A52

15

Epi#24

D120, K27, A24, P86, F21, A18, K15

D14, K22, A24, P86, F21, A18, K15

D76, K22, A24, P86, S87, F21, K15

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Epi#25

R249, R247, E197, E195

Epi#27

25 D172, E195, E197, K265

E197, E195, D172, K136

D172, E197, E195, K170

Epi#28

30 A18, D14, Q19, K15, E271, K12, Q17, S87, D76

V4, D14, Q17, K12, E271, K15, F21, A18, K22

Epi#29

L257, K265, L196, F261, E195

35 G53, N97, L96, F50, E54

Epi#30

G146, L241, S242, H238, K237, P239, L235

G146, L241, S236, H238, S242, P239, L235

40

Epi#33

K15, F21, P86, S87, A24, K27

K27, Y91, V45, S89, A24, K22

45 Epi#34

V4, P5, T3, G80, P40, S38, T211

V108, W113, T116, G118, R145, Y143, S244

V26, P239, S242, G146, R145, T115, T116

50 Epi#36

296

A52, A56, A48, V51, G102, Y104, S105, V108, A138, A134
A52, A56, A48, V51, G102, Y104, S103, V108, A134, A138

Epi#37

5 Y262, A194, L196, K265, Y256
Y263, R186, L257, K265, Y256
Y256, A254, L257, K265, Y262

Epi#40

10 R186, L257, A254, Y256, K265, S252
R186, L257, G258, Y256, K265, S260

Epi#41

Y256, L257, S260
15 Y256, L257, S259

Epi#42

L235, P239, S242, N248, R249, Q275
L241, P239, S242, Q245, R249, Q275

20

Epi#44

S132, Q137, D140, Y143, A144, A138, T133
V108, Q137, D140, Y143, A144, A138, T133
S173, Q137, D140, Y143, A144, A138, T133

25

Epi#48

Q19, K15, P9, P5, V4
E271, K15, P9, P5, V4

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Protease B:

Epi#05

SAS: 454, Size 24.86: G189, A188, R164, P127, G125, S99
35 SAS: 452, Size 15.92: G189, A188, R164, P127, G125, S128
SAS: 451, Size 24.86: G157, A188, R164, P127, G125, S99
SAS: 449, Size 15.92: G157, A188, R164, P127, G125, S128
SAS: 445, Size 23.31: G189, A166, R164, P127, G125, S99

40 Epi#09

SAS: 446, Size 15.76: T254, G189, A166, R164, A188, S158
SAS: 312, Size 15.90: T22, G20, L21, R19, A15, S9

Epi#10

45 SAS: 460, Size 17.32: D175, N177, N179, S182, F183, G155, R180
SAS: 437, Size 16.70: D211, N212, N153, S182, F183, G155, R180
SAS: 424, Size 13.75: D175, N212, N153, S182, F183, G155, R180
SAS: 417, Size 16.70: D211, N212, N153, S154, F183, G155, R180
SAS: 404, Size 15.83: D175, N212, N153, S154, F183, G155, R180

50

Epi#12

SAS: 309, Size 13.46: P127, Y161, E134, P129
SAS: 292, Size 9.37: R164, Y161, E134, P129
SAS: 287, Size 18.66: P127, Y161, E134, N138
5 SAS: 284, Size 16.85: P127, Y161, E134, N167
SAS: 275, Size 11.53: S128, Y161, E134, P129

Epi#17

SAS: 275, Size 15.84: A188, S158, R164, S126
10 SAS: 225, Size 12.79: A156, S158, R164, S126

Epi#18

SAS: 444, Size 16.32: S250, K245, S259, L256, A188, T254, L251
SAS: 397, Size 14.14: S250, K245, S259, L256, G252, T254, L251
15 SAS: 397, Size 14.14: S250, K245, S259, L251, G252, T254, L256
SAS: 397, Size 14.14: S259, K245, S250, L251, G252, T254, L256
SAS: 396, Size 21.52: S158, R164, S126, V102, G100, S99, L124

Epi#19

20 SAS: 295, Size 15.06: D175, W6, S9, Q12, R10
SAS: 278, Size 21.23: E110, T141, S236, Q239, R241

Epi#23

SAS: 486, Size 19.88: R143, N114, E110, S139, Q135, A131
25 SAS: 473, Size 18.68: R19, N18, E265, L21, Q230, P233
SAS: 468, Size 15.74: R164, N167, E134, S139, Q135, A131
SAS: 463, Size 13.77: R164, N167, E134, S130, Q135, A131
SAS: 461, Size 21.98: R44, N42, E87, S24, Q230, P233

30 Epi#28

SAS: 520, Size 19.27: V102, Q107, W111, E110, Q135, S139, R143
SAS: 492, Size 24.70: V102, Q107, F49, E53, Q57, G46, R44
SAS: 480, Size 22.76: V50, Q107, W111, E110, Q135, S139, R143
SAS: 452, Size 19.08: V50, Q107, F49, E53, Q57, G46, R44
35 SAS: 441, Size 24.70: V102, Q107, E110, W111, F49, G46, R44

Epi#29

SAS: 239, Size 11.49: G20, N18, L21, E265
SAS: 224, Size 11.49: G20, R19, L21, E265
40 SAS: 179, Size 16.62: I4, P14, L21, E265
SAS: 175, Size 11.49: G20, K231, L21, E265
SAS: 153, Size 18.96: G25, Q230, L21, E265

Epi#30

45 SAS: 308, Size 24.27: G20, L21, A15, H17, S85, L73, P39

Epi#31

SAS: 363, Size 21.72: L256, R180, N178, R10, W6, V197, D211
SAS: 352, Size 22.95: L251, R180, N178, R10, W6, V197, D211
50 SAS: 350, Size 21.62: L256, R180, N178, R10, W6, V197, D175

298

SAS: 339, Size 17.75: L251, R180, N178, R10, W6, V197, D175

Epi#34

SAS: 430, Size 18.33: V238, W235, S236, G144, R143, S139, S142
5 SAS: 430, Size 18.33: V238, W235, S236, G144, R143, S142, S139
SAS: 420, Size 13.98: V238, W235, S236, G144, R143, S142, T141
SAS: 420, Size 13.98: V238, W235, S236, G144, R143, T141, S142
SAS: 352, Size 18.33: V238, W235, S236, G144, R143, S139, T141

10 Epi#37

SAS: 415, Size 23.06: T254, A188, L256, R180, N177
SAS: 374, Size 18.08: T254, A188, L256, R180, N179
SAS: 335, Size 19.96: T254, A188, L256, R180, N178

15 Epi#39

SAS: 425, Size 16.00: A166, E134, R164, P127, G125, L124
SAS: 421, Size 16.36: A131, E134, R164, P127, G125, L124
SAS: 400, Size 16.00: A166, E134, R164, P129, G125, L124
SAS: 396, Size 16.36: A131, E134, R164, P129, G125, L124
20 SAS: 359, Size 16.00: A166, E134, T132, P129, G125, L124

Epi#40

SAS: 358, Size 15.76: A166, G189, Y186, A188, T254
SAS: 352, Size 15.76: A166, G189, T254, A188, S158
25 SAS: 326, Size 11.62: A96, G59, T56, P54, S55
SAS: 322, Size 15.30: G98, G59, T56, P54, S55
SAS: 318, Size 17.81: A188, G189, Y186, A156, S182

Epi#42

30 SAS: 528, Size 16.22: L21, P14, S9, Q12, H17, R19, R269

Epi#44

SAS: 401, Size 15.10: L256, R180, Y186, S158, A188, T254
SAS: 393, Size 15.52: L256, R180, Y186, A188, G189, T254
35 SAS: 390, Size 18.46: L251, R180, Y186, S158, A188, T254
SAS: 382, Size 16.23: L251, R180, Y186, A188, G189, T254
SAS: 376, Size 22.23: V197, R180, Y186, S158, A188, T254

Epi#46

40 SAS: 559, Size 12.63: A15, R269, R19, P14, N18, G20

Epi#53

SAS: 298, Size 9.48: W235, S234, Q230, K231
SAS: 298, Size 18.05: W235, S234, Q239, K245
45 SAS: 289, Size 9.48: W235, P233, Q230, K231
SAS: 283, Size 9.61: W235, S234, Q239, K229
SAS: 255, Size 14.51: W235, S236, Q239, K245

50 ProteaseC:

Epi#05

SAS: 445, Size 23.34: G189, A166, R164, P127, G125, S99
SAS: 445, Size 24.90: G189, A188, R164, P127, G125, S99
SAS: 433, Size 24.90: G157, A188, R164, P127, G125, S99
5 SAS: 427, Size 15.89: G189, A188, R164, P127, G125, S128
SAS: 427, Size 15.50: G189, A166, R164, P127, G125, S128

Epi#09

SAS: 463, Size 15.74: T254, G189, A166, R164, A188, S158
10 SAS: 425, Size 15.74: D191, G189, A166, R164, A188, T254
SAS: 384, Size 13.57: D191, G189, A166, R164, A188, S158

Epi#10

SAS: 445, Size 17.28: D175, N177, N179, S182, F183, G155, R180
15 SAS: 431, Size 13.75: D175, N212, N153, S182, F183, G155, R180
SAS: 403, Size 15.83: D175, N212, N153, S154, F183, G155, R180
SAS: 387, Size 16.14: D175, N178, N179, S182, F183, G155, R180
SAS: 373, Size 16.76: D175, N212, N153, A156, F183, G155, R180

20 Epi#12

SAS: 292, Size 13.45: P127, Y161, E134, P129
SAS: 287, Size 9.30: R44, Y89, E87, N42
SAS: 284, Size 9.35: R164, Y161, E134, P129
SAS: 282, Size 9.35: R164, Y165, E134, P129
25 SAS: 272, Size 16.85: P127, Y161, E134, N167

Epi#16

SAS: 547, Size 20.59: R164, P129, Y165, G189, S158, N255, D191,
A166, N167
30 SAS: 543, Size 23.80: R164, P129, Y165, G189, S158, N255, D191,
A166, N138

Epi#17

SAS: 267, Size 15.84: A188, S158, R164, S126
35 SAS: 231, Size 12.82: A156, S158, R164, S126

Epi#18

SAS: 449, Size 16.85: S182, R180, L256, A188, T254, L251
SAS: 426, Size 21.97: S126, R164, S158, A188, T254, L256
40 SAS: 407, Size 15.92: S182, R180, L251, G252, T254, L256
SAS: 407, Size 15.92: S182, R180, L256, G252, T254, L251
SAS: 391, Size 18.26: S182, R180, L256, G252, S250, L251

Epi#19

45 SAS: 293, Size 15.04: D175, W6, S9, Q12, R10
SAS: 291, Size 17.13: D191, N242, S236, Q239, R241
SAS: 273, Size 21.24: E110, T141, S236, Q239, R241

Epi#23

50 SAS: 463, Size 19.84: R143, N114, E110, S139, Q135, A131

300

SAS: 451, Size 15.68: R164, N167, E134, S139, Q135, A131
SAS: 443, Size 21.95: R44, N42, E87, S24, Q230, P233
SAS: 440, Size 22.70: R143, N115, E110, S139, Q135, A131
SAS: 431, Size 15.11: R44, N42, E87, S85, L73, P39

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Epi#28

SAS: 402, Size 18.79: G59, Q57, E53, F49, G46, R44
SAS: 384, Size 20.81: A96, Q57, E53, F49, G46, R44
SAS: 376, Size 18.79: A47, Q57, E53, F49, G46, R44

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Epi#31

SAS: 348, Size 21.63: L256, R180, N178, R10, W6, V197, D175
SAS: 342, Size 17.75: L251, R180, N178, R10, W6, V197, D175

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Epi#33

SAS: 399, Size 18.88: Q107, Y102, P129, S126, R164
SAS: 355, Size 15.95: Q135, Y165, P129, S126, R164

Epi#34

20 SAS: 424, Size 18.37: V238, W235, S236, G144, R143, S139, S142
SAS: 424, Size 18.37: V238, W235, S236, G144, R143, S142, S139
SAS: 408, Size 14.02: V238, W235, S236, G144, R143, S142, T141
SAS: 408, Size 14.02: V238, W235, S236, G144, R143, T141, S142
SAS: 346, Size 18.37: V238, W235, S236, G144, R143, T141, S139

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Epi#37

SAS: 405, Size 23.05: T254, A188, L256, R180, N177
SAS: 364, Size 18.08: T254, A188, L256, R180, N179
SAS: 347, Size 19.96: T254, A188, L256, R180, N178

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Epi#40

SAS: 368, Size 15.74: A166, G189, T254, A188, S158
SAS: 362, Size 15.74: A166, G189, Y186, A188, T254
SAS: 326, Size 17.80: A188, G189, Y186, A156, S182
35 SAS: 326, Size 23.72: A166, G189, Y186, A156, S182
SAS: 326, Size 17.80: G189, A188, Y186, A156, S182

Epi#41

SAS: 232, Size 19.49: P204, Y208, L211, V197, S210

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Epi#44

SAS: 445, Size 22.71: V238, R241, D191, Y186, S158, A188, T254
SAS: 429, Size 21.14: V238, R241, D191, Y186, A188, G189, T254
SAS: 410, Size 22.71: V238, R241, D191, Y186, S158, G189, T254
45 SAS: 404, Size 23.33: V238, R241, D191, Y257, S250, G252, T254
SAS: 382, Size 23.33: V238, R241, D191, Y257, S253, G252, T254

Epi#46

SAS: 567, Size 12.67: A15, R269, R19, P14, N18, G20

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301

Epi#53

SAS: 305, Size 9.43: W235, S234, Q230, K231
SAS: 303, Size 9.53: W235, S234, Q239, K229
SAS: 276, Size 9.43: W235, P233, Q230, K231
5 SAS: 259, Size 9.43: W235, S234, Q230, K229
SAS: 233, Size 9.53: W235, S236, Q239, K229

ProteaseD:

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Epi#05

SAS: 453, Size 24.94: G189, A188, R164, P127, G125, S99
SAS: 449, Size 23.37: G189, A166, R164, P127, G125, S99
SAS: 442, Size 24.94: G157, A188, R164, P127, G125, S99
15 SAS: 439, Size 15.91: G189, A188, R164, P127, G125, S128
SAS: 435, Size 15.50: G189, A166, R164, P127, G125, S128

Epi#09

SAS: 448, Size 15.77: T254, G189, A166, R164, A188, S158

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Epi#10

SAS: 460, Size 17.32: D175, N177, N179, S182, F183, G155, R180
SAS: 428, Size 13.76: D175, N212, N153, S182, F183, G155, R180
SAS: 403, Size 15.83: D175, N212, N153, S154, F183, G155, R180
25 SAS: 391, Size 16.15: D175, N178, N179, S182, F183, G155, R180
SAS: 372, Size 16.77: D175, N212, N153, A156, F183, G155, R180

Epi#12

SAS: 302, Size 13.47: P127, Y161, E134, P129
30 SAS: 290, Size 9.39: R164, Y161, E134, P129
SAS: 282, Size 18.68: P127, Y161, E134, N138
SAS: 280, Size 16.87: P127, Y161, E134, N167
SAS: 270, Size 13.10: R164, Y161, E134, N138

35 Epi#17

SAS: 286, Size 15.87: A188, S158, R164, S126
SAS: 250, Size 12.76: A156, S158, R164, S126

Epi#18

40 SAS: 446, Size 16.31: S250, K245, S259, L256, A188, T254, L251
SAS: 406, Size 14.13: S250, K245, S259, L256, G252, T254, L251
SAS: 406, Size 14.13: S250, K245, S259, L251, G252, T254, L256
SAS: 406, Size 14.13: S259, K245, S250, L251, G252, T254, L256
SAS: 388, Size 14.13: S250, K245, S259, L256, G252, T249, L251

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Epi#19

SAS: 319, Size 15.07: D175, W6, S9, Q12, R10
SAS: 276, Size 21.28: E110, T141, S236, Q239, R241

50 Epi#23

302

SAS: 497, Size 19.86: R143, N114, E110, S139, Q135, A131
SAS: 487, Size 15.77: R164, N167, E134, S139, Q135, A131
SAS: 478, Size 13.78: R164, N167, E134, S130, Q135, A131
SAS: 477, Size 18.16: R143, N138, E134, S139, Q135, A131
5 SAS: 472, Size 22.70: R143, N115, E110, S139, Q135, A131

Epi#28

SAS: 554, Size 22.17: A101, Q107, I102, E134, Q135, S139, R143
SAS: 532, Size 19.36: I102, Q107, W111, E110, Q135, S139, R143
10 SAS: 527, Size 22.79: V50, Q107, I102, E134, Q135, S139, R143
SAS: 509, Size 24.76: I102, Q107, F49, E53, Q57, G46, R44
SAS: 508, Size 22.17: A101, Q107, W111, E110, Q135, S139, R143

Epi#31

15 SAS: 355, Size 21.56: L256, R180, N178, R10, W6, V197, D175
SAS: 352, Size 17.71: L251, R180, N178, R10, W6, V197, D175

Epi#34

SAS: 457, Size 18.37: V238, W235, S236, G144, R143, S139, S142
20 SAS: 457, Size 18.37: V238, W235, S236, G144, R143, S142, S139
SAS: 447, Size 14.02: V238, W235, S236, G144, R143, S142, T141
SAS: 447, Size 14.02: V238, W235, S236, G144, R143, T141, S142
SAS: 374, Size 18.37: V238, W235, S236, G144, R143, T141, S139

25 Epi#37

SAS: 397, Size 23.08: T254, A188, L256, R180, N177
SAS: 361, Size 18.08: T254, A188, L256, R180, N179
SAS: 328, Size 19.98: T254, A188, L256, R180, N178

30 Epi#39

SAS: 425, Size 16.36: A131, E134, R164, P127, G125, L124
SAS: 423, Size 16.02: A166, E134, R164, P127, G125, L124
SAS: 399, Size 16.36: A131, E134, R164, P129, G125, L124
SAS: 397, Size 16.02: A166, E134, R164, P129, G125, L124
35 SAS: 379, Size 16.36: A131, E134, T132, P129, G125, L124

Epi#40

SAS: 354, Size 15.77: A166, G189, T254, A188, S158
SAS: 351, Size 15.77: A166, G189, Y186, A188, T254
40 SAS: 334, Size 17.81: G189, A188, Y186, A156, S182
SAS: 334, Size 17.81: A188, G189, Y186, A156, S182
SAS: 330, Size 14.42: A166, G189, Y186, A188, S158

Epi#41

45 SAS: 217, Size 19.46: P204, Y208, L211, V197, S210

Epi#44

SAS: 407, Size 15.10: L256, R180, Y186, S158, A188, T254
SAS: 404, Size 18.45: L251, R180, Y186, S158, A188, T254
50 SAS: 387, Size 15.52: L256, R180, Y186, A188, G189, T254

303

SAS: 384, Size 16.23: L251, R180, Y186, A188, G189, T254

SAS: 373, Size 22.26: V197, R180, Y186, S158, A188, T254

Epi#46

5 SAS: 545, Size 12.69: A15, R269, R19, P14, N18, G20

Epi#53

SAS: 306, Size 18.06: W235, S234, Q239, K245

SAS: 277, Size 9.52: W235, S234, Q239, K229

10 SAS: 276, Size 9.46: W235, S234, Q230, K231

SAS: 268, Size 9.46: W235, P233, Q230, K231

SAS: 258, Size 14.50: W235, S236, Q239, K245

15 **ProteaseE:**

Epi#05

SAS: 461, Size 15.49: G189, A166, R164, P127, G125, S128

SAS: 459, Size 15.90: G189, A188, R164, P127, G125, S128

20 SAS: 435, Size 15.49: G189, A166, R164, P127, G125, S126

SAS: 433, Size 15.49: G189, A166, R164, P129, G125, S128

SAS: 433, Size 15.86: G189, A188, R164, P127, G125, S126

Epi#06

25 SAS: 518, Size 14.10: G189, A188, D157, S158, R164, P127

SAS: 490, Size 15.98: G189, A188, D157, S158, R164, P129

SAS: 460, Size 14.60: G155, A156, D157, S158, R164, P127

SAS: 432, Size 17.71: G155, A156, D157, S158, R164, P129

30 Epi#09

SAS: 482, Size 15.78: T254, G189, A166, R164, A188, S158

SAS: 311, Size 15.91: T22, G20, L21, R19, A15, S9

Epi#10

35 SAS: 455, Size 17.26: D175, N177, N179, S182, F183, G155, R180

SAS: 406, Size 13.76: D175, N212, N153, S182, F183, G155, R180

SAS: 383, Size 16.16: D175, N178, N179, S182, F183, G155, R180

SAS: 381, Size 15.82: D175, N212, N153, S154, F183, G155, R180

SAS: 347, Size 16.78: D175, N212, N153, A156, F183, G155, R180

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Epi#12

SAS: 310, Size 13.48: P127, Y161, E134, P129

SAS: 306, Size 9.40: R164, Y161, E134, P129

SAS: 297, Size 9.40: R164, Y165, E134, P129

45 SAS: 285, Size 16.90: P127, Y161, E134, N167

SAS: 281, Size 18.68: P127, Y161, E134, N138

Epi#16

SAS: 673, Size 19.67: R164, P127, Y161, G125, S126, S154, D157,

50 A188, N255

304

- SAS: 664, Size 20.60: R164, P129, Y165, G189, S158, S154, D157, A188, N255
SAS: 645, Size 20.60: R164, P129, Y161, G125, S126, S154, D157, A188, N255
5 SAS: 636, Size 14.89: R164, P127, Y161, G125, S126, S154, D157, A156, N153
SAS: 627, Size 17.25: R164, P129, Y165, G189, S158, S154, D157, A156, N153
- 10 Epi#17
SAS: 305, Size 15.86: A188, S158, R164, S126
SAS: 270, Size 12.73: A156, S158, R164, S126
- Epi#18
15 SAS: 590, Size 17.32: S250, K246, S259, L256, A188, T254, L251
SAS: 551, Size 16.26: S259, K246, S250, L251, G252, T254, L256
SAS: 551, Size 16.26: S250, K246, S259, L251, G252, T254, L256
SAS: 551, Size 16.26: S250, K246, S259, L256, G252, T254, L251
SAS: 518, Size 16.26: S250, K246, S259, L251, G252, S253, L256
- 20 Epi#23
SAS: 471, Size 19.86: R143, N114, E110, S139, Q135, A131
SAS: 467, Size 13.75: R164, N167, E134, S130, Q135, A131
SAS: 467, Size 15.76: R164, N167, E134, S139, Q135, A131
25 SAS: 451, Size 22.69: R143, N115, E110, S139, Q135, A131
SAS: 446, Size 19.99: R143, N138, E134, S130, Q135, A131
- Epi#28
SAS: 505, Size 19.43: I102, Q107, W111, E110, Q135, S139, R143
30 SAS: 500, Size 22.22: A101, Q107, W111, E110, Q135, S139, R143
SAS: 499, Size 24.79: I102, Q107, F49, E53, Q57, G46, R44
SAS: 494, Size 24.56: A101, Q107, F49, E53, Q57, G46, R44
SAS: 441, Size 24.79: I102, Q107, E110, W111, F49, G46, R44
- 35 Epi#29
SAS: 216, Size 9.94: I43, R44, L41, E87
SAS: 209, Size 10.85: L73, N42, L41, E87
SAS: 200, Size 13.98: G46, R44, L41, E87
SAS: 199, Size 11.98: G45, R44, L41, E87
40 SAS: 197, Size 19.08: I77, N74, L41, E87
- Epi#30
SAS: 318, Size 24.25: G20, L21, A15, H17, S85, L73, P39
SAS: 277, Size 24.25: G20, L21, A15, H17, S85, L41, P39
45 SAS: 258, Size 21.05: G20, L21, A15, H17, S85, L73, L41
- Epi#31
SAS: 377, Size 21.62: L256, R180, N178, R10, W6, V197, D175
SAS: 370, Size 17.72: L251, R180, N178, R10, W6, V197, D175

Epi#33

SAS: 388, Size 15.92: Q135, Y165, P129, S126, R164

Epi#34

5 SAS: 420, Size 18.35: V238, W235, S236, G144, R143, S139, S142
SAS: 411, Size 13.98: V238, W235, S236, G144, R143, S142, T141
SAS: 341, Size 18.35: V238, W235, S236, G144, R143, S139, T141

Epi#37

10 SAS: 412, Size 23.05: T254, A188, L256, R180, N177
SAS: 378, Size 18.07: T254, A188, L256, R180, N179
SAS: 340, Size 20.00: T254, A188, L256, R180, N178

Epi#39

15 SAS: 445, Size 16.04: A166, E134, R164, P127, G125, L124
SAS: 432, Size 16.40: A131, E134, R164, P127, G125, L124
SAS: 417, Size 16.04: A166, E134, R164, P129, G125, L124
SAS: 404, Size 16.40: A131, E134, R164, P129, G125, L124
SAS: 376, Size 16.04: A166, E134, T132, P129, G125, L124

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Epi#40

SAS: 374, Size 15.78: A166, G189, T254, A188, S158
SAS: 334, Size 15.78: A166, G189, Y186, A188, T254
SAS: 317, Size 11.62: A96, G59, T56, P54, S55
25 SAS: 312, Size 15.30: G98, G59, T56, P54, S55
SAS: 307, Size 15.49: G189, A166, Y165, P129, S128

Epi#41

SAS: 234, Size 19.50: P204, Y208, L211, V197, S210
30 SAS: 189, Size 19.50: P204, Y208, L211, V197, S215

Epi#42

SAS: 549, Size 16.42: L21, P14, S9, Q12, H17, R19, R269

Epi#44

35 SAS: 398, Size 15.10: L256, R180, Y186, S158, A188, T254
SAS: 391, Size 18.47: L251, R180, Y186, S158, A188, T254
SAS: 372, Size 15.51: L256, R180, Y186, A188, G189, T254
SAS: 371, Size 12.26: L256, R180, Y257, S250, G252, T254
40 SAS: 367, Size 15.51: L256, R180, Y186, S158, G189, T254

Epi#46

SAS: 575, Size 12.75: A15, R269, R19, P14, N18, G20

Epi#47

45 SAS: 491, Size 19.28: G45, E87, I43, R44, L41, N42, P39, S206

Epi#53

SAS: 202, Size 9.12: W235, P233, K231
50 SAS: 199, Size 9.12: W235, S234, K231

306

SAS: 182, Size 6.73: W235, P233, K229
 SAS: 179, Size 7.76: W235, S234, K229
 SAS: 131, Size 8.39: W235, S236, K229

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Properase:**Epi#05**

SAS: 456, Size 15.94: G189, A188, R164, P127, G125, S128
 10 SAS: 453, Size 15.52: G189, A166, R164, P127, G125, S128
 SAS: 451, Size 15.94: G157, A188, R164, P127, G125, S128
 SAS: 427, Size 15.94: G189, A188, R164, P129, G125, S128
 SAS: 424, Size 15.52: G189, A166, R164, P129, G125, S128

15 **Epi#09**

SAS: 480, Size 15.73: T254, G189, A166, R164, A188, S158
 SAS: 302, Size 15.88: T22, G20, L21, R19, A15, S9

Epi#10

20 SAS: 470, Size 17.27: D175, N177, N179, S182, F183, G155, R180
 SAS: 446, Size 13.75: D175, N212, N153, S182, F183, G155, R180
 SAS: 420, Size 15.84: D175, N212, N153, S154, F183, G155, R180
 SAS: 396, Size 16.09: D175, N178, N179, S182, F183, G155, R180
 SAS: 380, Size 16.78: D175, N212, N153, A156, F183, G155, R180

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Epi#12

SAS: 296, Size 9.36: R164, Y161, E134, P129
 SAS: 295, Size 13.45: P127, Y161, E134, P129
 SAS: 291, Size 9.36: R164, Y165, E134, P129
 30 SAS: 271, Size 14.70: R164, Y161, E134, N102
 SAS: 270, Size 13.45: P127, Y161, E134, N102

Epi#17

SAS: 283, Size 15.87: A188, S158, R164, S126
 35 SAS: 241, Size 12.73: A156, S158, R164, S126

Epi#18

SAS: 474, Size 16.26: S250, K245, S259, L256, A188, T254, L251
 SAS: 435, Size 14.14: S250, K245, S259, L256, G252, T254, L251
 40 SAS: 398, Size 14.14: S259, K245, S250, L251, G252, S253, L256

Epi#19

SAS: 260, Size 21.26: E110, T141, S236, Q239, R241

45 **Epi#23**

SAS: 491, Size 19.86: R143, N114, E110, S139, Q135, A131
 SAS: 482, Size 15.76: R164, N167, E134, S139, Q135, A131
 SAS: 465, Size 22.69: R143, N115, E110, S139, Q135, A131
 SAS: 462, Size 18.17: R143, N138, E134, S139, Q135, A131
 50 SAS: 439, Size 18.17: R143, N138, E110, S139, Q135, A131

Epi#28
SAS: 445, Size 22.79: V50, Q107, W111, E110, Q135, S139, R143
SAS: 426, Size 19.06: V50, Q107, F49, E53, Q57, G46, R44
5 SAS: 370, Size 19.06: V50, Q107, E110, W111, F49, G46, R44

Epi#31
SAS: 347, Size 21.62: L256, R180, N178, R10, W6, V197, D175
SAS: 339, Size 17.74: L251, R180, N178, R10, W6, V197, D175
10

Epi#33
SAS: 368, Size 15.95: Q135, Y165, P129, S126, R164

Epi#34
15 SAS: 445, Size 18.39: V238, W235, S236, G144, R143, S139, S142
SAS: 436, Size 14.07: V238, W235, S236, G144, R143, S142, T141
SAS: 358, Size 18.39: V238, W235, S236, G144, R143, T141, S139

Epi#37
20 SAS: 415, Size 23.03: T254, A188, L256, R180, N177
SAS: 374, Size 18.04: T254, A188, L256, R180, N179
SAS: 341, Size 19.93: T254, A188, L256, R180, N178

Epi#39
25 SAS: 323, Size 11.55: A15, E265, H17, R19, P14, G20, L21
SAS: 238, Size 12.13: A15, E265, H17, T22, P14, G20, L21

Epi#40
30 SAS: 370, Size 15.73: A166, G189, T254, A188, S158
SAS: 360, Size 15.73: A166, G189, Y186, A188, T254
SAS: 324, Size 17.80: A188, G189, Y186, A156, S182
SAS: 321, Size 23.71: A166, G189, Y186, A156, S182

Epi#41
35 SAS: 228, Size 19.53: P204, Y208, L211, V197, S210

Epi#42
SAS: 554, Size 16.31: L21, P14, S9, Q12, H17, R19, R269

Epi#44
40 SAS: 406, Size 15.06: L256, R180, Y186, S158, A188, T254
SAS: 398, Size 18.38: L251, R180, Y186, S158, A188, T254
SAS: 395, Size 12.22: L256, R180, Y257, S250, G252, T254
SAS: 392, Size 15.49: L256, R180, Y186, A188, G189, T254
45 SAS: 387, Size 12.22: L251, R180, Y257, S250, G252, T254

Epi#46
SAS: 581, Size 12.65: A15, R269, R19, P14, N18, G20

50 Epi#53

308

SAS: 297, Size 18.06: W235, S234, Q239, K245
SAS: 283, Size 9.54: W235, S234, Q239, K229
SAS: 250, Size 9.46: W235, S234, Q230, K231
SAS: 249, Size 14.49: W235, S236, Q239, K245
5 SAS: 247, Size 9.46: W235, P233, Q230, K231

Release:

10 Epi#05

SAS: 461, Size 17.25: G158, A189, R165, P128, G126, S129
SAS: 439, Size 17.22: G158, A189, R165, P128, G126, S127
SAS: 436, Size 17.25: G158, A189, S159, P128, G126, S129
SAS: 420, Size 17.25: G158, A189, R165, P130, G126, S129
15 SAS: 414, Size 17.22: G158, A189, S159, P128, G126, S127

Epi#09

SAS: 510, Size 22.37: T22, G20, R19, A15, R270, A267, T250
SAS: 501, Size 22.37: L21, G20, R19, A15, R270, A267, T250

20

Epi#10

SAS: 458, Size 17.50: D176, N178, N180, S183, F184, G156, R181
SAS: 424, Size 13.68: D176, N213, N154, S183, F184, G156, R181
SAS: 407, Size 15.87: D176, N213, N154, S155, F184, G156, R181
25 SAS: 392, Size 16.18: D176, N179, N180, S183, F184, G156, R181
SAS: 362, Size 16.73: D176, N213, N154, A157, F184, G156, R181

Epi#12

SAS: 323, Size 9.38: R45, Y90, E88, N43
30 SAS: 312, Size 13.53: P128, Y162, E135, P130
SAS: 302, Size 9.46: R165, Y162, E135, P130
SAS: 296, Size 9.46: R165, Y166, E135, P130
SAS: 295, Size 13.19: T255, Y187, E190, S159

35 Epi#18

SAS: 431, Size 15.20: S251, K246, S260, L257, A189, T255, L252
SAS: 398, Size 14.35: S251, K246, S260, L252, G253, T255, L257
SAS: 378, Size 14.35: S251, K246, S260, L257, G253, T250, L252

40 Epi#19

SAS: 285, Size 21.53: E111, T142, S237, Q240, R242
SAS: 275, Size 12.58: D119, T142, S237, Q240, R242

Epi#23

45 SAS: 512, Size 22.29: R45, N43, E88, S24, Q231, P234
SAS: 476, Size 19.71: R144, N115, E111, S140, Q136, A132
SAS: 460, Size 13.83: R165, N168, E135, S131, Q136, A132
SAS: 455, Size 20.11: R144, N139, E135, S131, Q136, A132
SAS: 452, Size 15.83: R165, N168, E135, S140, Q136, A132

50

Epi#25

SAS: 293, Size 13.93: R45, K27, D119, E88

Epi#28

5 SAS: 502, Size 19.99: V103, Q108, W112, E111, Q136, S140, R144
SAS: 476, Size 21.74: V51, Q108, F50, E54, Q58, S37, R45
SAS: 472, Size 24.93: V103, Q108, F50, E54, Q58, G47, R45
SAS: 469, Size 23.18: V51, Q108, W112, E111, Q136, S140, R144
SAS: 439, Size 19.16: V51, Q108, F50, E54, Q58, G47, R45

10

Epi#31

SAS: 354, Size 21.73: L257, R181, N179, R10, W6, V198, D176
SAS: 348, Size 17.85: L252, R181, N179, R10, W6, V198, D176

15

Epi#33

SAS: 396, Size 22.75: Q201, Y204, P205, S37, R45
SAS: 379, Size 22.75: Q201, Y209, P205, S37, R45
SAS: 357, Size 18.39: H63, Y204, P205, S37, R45

20

Epi#34

SAS: 466, Size 13.97: V239, W236, S237, G145, R144, S143, T142
SAS: 463, Size 18.37: V239, W236, S237, G145, R144, S140, S143
SAS: 387, Size 18.37: V239, W236, S237, G145, R144, S140, T142

25

Epi#36

SAS: 206, Size 22.37: T250, A267, A15, G20, T22

Epi#37

SAS: 400, Size 22.59: T255, A189, L257, R181, N178
30 SAS: 359, Size 17.59: T255, A189, L257, R181, N180
SAS: 334, Size 19.35: T255, A189, L257, R181, N179

Epi#39

SAS: 464, Size 16.36: A167, E135, R165, P128, G126, L125
35 SAS: 444, Size 16.52: A132, E135, R165, P128, G126, L125
SAS: 441, Size 16.36: A167, E190, R165, P128, G126, L125
SAS: 441, Size 18.98: A189, E190, R165, P128, G126, L125
SAS: 423, Size 16.36: A167, E135, R165, P130, G126, L125

40

Epi#40

SAS: 324, Size 11.66: A97, G60, T57, P55, S56
SAS: 316, Size 17.09: G158, A189, Y187, A157, S183
SAS: 307, Size 14.92: G158, A157, Y187, A189, T255
SAS: 307, Size 15.34: G99, G60, T57, P55, S56

45

Epi#41

SAS: 222, Size 19.74: P205, Y209, L212, V198, S211

Epi#42

50 SAS: 544, Size 16.22: L21, P14, S9, Q12, H17, R19, R270

Epi#44

SAS: 421, Size 14.87: L257, R181, Y187, S159, A189, T255
 SAS: 415, Size 18.81: L252, R181, Y187, S159, A189, T255
 5 SAS: 389, Size 22.36: V198, R181, Y187, S159, A189, T255
 SAS: 389, Size 21.81: I44, R45, Y90, A48, V51, P52
 SAS: 386, Size 19.16: I44, R45, Y90, A48, V51, P55

Epi#46

10 SAS: 557, Size 14.54: A267, R270, R19, P14, N18, G20
 SAS: 553, Size 12.63: A15, R270, R19, P14, N18, G20
 SAS: 540, Size 13.10: A267, R270, R19, P14, N18, A15
 SAS: 444, Size 14.54: A267, R270, R19, P14, G20, A15

15 Epi#47

SAS: 627, Size 16.22: A267, R270, A15, R19, L21, N18, P14, S9
 SAS: 436, Size 15.11: A267, E266, A15, R19, L21, N18, P14, S9

Epi#51

20 SAS: 545, Size 21.66: L21, R19, H17, D75, S77, I78, S3, W6
 SAS: 485, Size 21.66: L21, R19, H17, D75, Q2, I78, S3, W6

Epi#53

SAS: 328, Size 9.43: W236, S235, Q231, K232
 25 SAS: 316, Size 9.43: W236, P234, Q231, K232
 SAS: 301, Size 18.21: W236, S235, Q240, K246
 SAS: 246, Size 14.68: W236, S237, Q240, K246

30 "SAS" is solvent accessible surface. "Size" is the total surface
 area of the epitope in Å².

35 Example 12

The object of this example is to provide evidence showing that
 subtilisins with an homology to BPN' of as low as 44,8% reveal a
 similar epitope distribution as BPN'.

40

Alcalase, Protease B, Savinase, Esperase, and PD498 (which range
 from 44,8% to 69,5% in sequence identity to BPN') were epitope

mapped as described in the above example, and compared with epitope mapped BPN' (Figure 1).

The data in Figure 1 show a significant overlap between the areas on the primary structure of the respective proteases. Overall, 6 regions were identified: 1-20, 35-65, 95-115, 130-145, 170-220, and 260-270.

Even better overlap between the epitope sequences can be found among proteins of higher sequence identity, such as within the Savinase-like subtilisins with more than 81% identity, preferably more than 85%, more preferably more than 90%, even more preferably more than 96% or most preferably more than 98% identity.

15

Example 13

Wash performance

20

The following example provides results from a number of washing tests that were conducted under the conditions indicated

Table 9: Experimental conditions for evaluation of Subtilisin variants I44V.

Detergent	OMO Acao
Detergent dose	2.5 g/l
PH	10.5
Wash time	14 min.

Temperature	25°C
Water hardness	9°dH
Enzymes	Subtilisin variant I44V
Enzyme conc.	10 nM
Test system	150 ml glass beakers with a stirring rod
Textile/volume	5 textile pieces (Ø 2.5 cm) in 50 ml detergent
Test material	EMPA117 from Center for Testmaterials, Holland

Table 10: Experimental conditions for evaluation of Subtilisin variants Q12D.

5

Detergent	Persil Powder
Detergent dose	4 g/l
PH	10.5
Wash time	20 min.
Temperature	30°C
Water hardness	18°dH
Enzymes	Subtilisin variant Q12D
Enzyme conc.	10 nM
Test system	150 ml glass beakers with a stirring rod

Textile/volume	5 textile pieces (Ø 2.5 cm) in 50 ml detergent
Test material	EMPA116 from Center for Testmaterials, Holland

Table 11: Experimental conditions for evaluation of Subtilisin variants Q12D.

5

Detergent	Tide
Detergent dose	1 g/l
PH	10.5
Wash time	10 min.
Temperature	25°C
Water hardness	6°dH
Enzymes	Subtilisin variant Q12D
Enzyme conc.	10 nM
Test system	150 ml glass beakers with a stirring rod
Textile/volume	5 textile pieces (Ø 2.5 cm) in 50 ml detergent
Test material	EMPA117 from Center for Testmaterials, Holland

pH is adjusted to 10.5 which is within the normal range for a powder detergent.

Water hardness was adjusted by adding CaCl_2 and MgCl_2 ($\text{Ca}^{2+}:\text{Mg}^{2+}$ = 2:1) to deionized water (see also Surfactants in Consumer Products - Theory, Technology and Application, Springer Verlag 1986). pH of the detergent solution was adjusted to pH 10.5 by addition of HCl.

Measurement of reflectance (R) on the test material was done at 460 nm using a Macbeth ColorEye 7000 photometer. The measurements were done according to the manufacturers protocol. The wash performance of the variants were evaluated by calculating a performance factor:

15

$$P = \frac{R_{\text{Variant}} - R_{\text{Blank}}}{R_{\text{Savinase}} - R_{\text{Blank}}}$$

P: Performance factor

R_{Variant} : Reflectance of test material washed with variant

20 R_{Savinase} : Reflectance of test material washed with Savinase[®]

R_{Blank} : Reflectance of test material washed with no enzyme

The variants all have improved wash performance compared to Savinase[®] - i.e. $P > 1$.

25 The variants can be divided into improvement classes designated with capital letters:

Class A: $1 < P \leq 1.5$

Class B: $1.5 < P \leq 2$

30 Class C: $P > 2$

Table 12: Subtilisin variants and improvement classes.

Improvement class	Variants
C	I44V, Q12D

As it can be seen from Table 12 SAVINASE® variants of the invention exhibits an improvement in wash performance.

Appendix A**Source code for the core C program (epitope.c)**

```

5  /* This is epitope.c */
   /* EPF 25-10-2000 */

10 /* ----- DEFINES ----- */

   #define MAXRESIDUES    1000
   #define MAXCONSENSUS   15
15  #define MAXEPITOPES    30000
   #define MAXEPITOPES    10000
   #define AMINOACIDS     "ACDEFGHIKLMNPQRSTVWY"
   #define AMINOACIDS3     "ALA CYS ASP GLU PHE GLY HIS ILE LYS LEU MET ASN PRO GLN ARG
SER THR VAL TRP TYR "
20  #define REVISIONDATE    "12-02-2001"
   #define max(A, B)      ((A) > (B) ? (A) : (B))
   #define min(A, B)      ((A) < (B) ? (A) : (B))

   /* ----- INCLUDES ----- */

25  #include <stdio.h>
   #include <stdlib.h>
   #include <string.h>
   #include <math.h>
30  #include <limits.h>

   /* ----- STRUCTS ----- */

   struct residue
35  {
       char ltr3[3];
       char ltr;
       float x, y, z;
       int sasa, number;
40  int member_of_epitopes;    /* how many epitopes is this residue part of ? */
   };

   struct epitoperesidue
   {
45  int parent;    /* -1 if top level */
       int residue;    /* -1 if gap */
       char level;
   };

50  struct epitope
   {
       int sasa, gaps, residues, res[MAXCONSENSUS];
       char epi[255];
       char subset;    /* is this epitope a subset of another */
55  float size;
   };

   /* ----- GLOBALS ----- */

60  struct residue res[MAXRESIDUES];
   struct epitoperesidue epires[MAXEPITOPES];
   char consensus[MAXCONSENSUS][22];
   struct epitope epi[MAXEPITOPES];
65  int numofres = 0, numofepires = 0, consensuslength = 0;
   int minsasa = 0, numofepitopes = 0, numofsubsets = 0;

```

317

```

float mindist = 7, sqmindist, maxsize, sqmaxsize, minlength = 0;
int maxepi = 0, minlength_residues, longestepitope;

5
/* ----- FILE FUNCTIONS ----- */

int readconsensus(char *filename)
10 {
    /* return length of consensus sequence */

    int i = 0;
    FILE *infile;
15    char buffer[255], end = 0;

    if (infile = fopen(filename, "r"))
    {
20 /*      This code adds linefeeds to the consensus file. This is because there must
        be a newline after the last line. Because of permission problems, this has
        been moved to
        the wrapping cgi-script instead

25    fclose(infile);
        infile = fopen(filename, "a");
        fprintf(infile, "\n\n");
        fclose(infile);
        infile = fopen(filename, "r");
30 */

        while (!feof(infile) && !end)
        {
            fgets (buffer, 255, infile);
35            if (strlen(buffer) > 22)
            {
                printf ("Too many residue types in consensus residue %d\n", i+1);
                printf ("using all 20 types instead.\n");
                strcpy (consensus[i], AMINOACIDS);
40            }
            else if (strchr(buffer, '*')) /* wildcard '*' means any residue, but no gap
*/
                strcpy (consensus[i], AMINOACIDS);
            else if (strchr(buffer, '?')) /* wildcard '?' means any residue or gap */
45            {
                strcpy (consensus[i], AMINOACIDS);
                strcat (consensus[i], "-");
            }
            else if (!strpbrk(buffer, "ACDEFGHIKLMNPQRSTVWY*?")) /* empty line, end the
50 loop */
            {
                end = 1;
                i--;
            }
            else
55            {
                strncpy (consensus[i], buffer, strlen(buffer)-1);
                i++;
            }
        }
60    fclose(infile);
        consensuslength = i;
        return i;
    }
65

int readpdbCA(char *filename)

```

318

```

{
    /* return number of residues */

    int i = 0;
    char *j;
5   FILE *infile;
    char buffer[255];
    char aminoacids[20] = AMINOACIDS;
    char aminoacids3[80] = AMINOACIDS3;
10
    if (infile = fopen(filename, "r"))
    {
        while (!feof(infile))
        {
15         fgets (buffer, 255, infile);
            if (!strcmp(buffer, "ATOM", 4) && !strcmp(buffer+13, "CA", 2)) /* get only the
CA atoms */
            {
                strncpy(res[i].ltr3, buffer+17, 3);
20         if (j = strstr(aminoacids3, res[i].ltr3))
                    res[i].ltr = aminoacids[(j-aminoacids3)/4];
                else
                {
                    printf("Unknown residue type: %s\n", res[i].ltr3);
25         res[i].ltr = 'X';
                }
                res[i].x = atof(buffer+30);
                res[i].y = atof(buffer+38);
                res[i].z = atof(buffer+46);
30         res[i].member_of_epitopes = 0;
                res[i].number = atoi(buffer+22);
                i++;
            }
        }
35     }
    numofres = i;
    return i;
}

40 int readresp(char *filename)
{
    /* return number of residues */

45     int i = 0;
    char *j;
    FILE *infile;
    char buffer[255];

50     strcpy (buffer, " ");

    if (infile = fopen(filename, "r"))
    {
        while (!feof(infile) && strcmp(buffer, " # RESIDUE AA", 15)) /* find where
55 data begins */
            fgets (buffer, 255, infile);

        while (!feof(infile))
        {
60         fgets (buffer, 255, infile);
            if (!feof(infile))
            {
                if ((buffer[13] == res[i].ltr && atoi(buffer+5) == res[i].number
)|| (strchr("abcdefghijklmnopqrstuvwxyz", buffer[13]) && res[i].ltr == 'C' &&
65 atoi(buffer+5) == res[i].number ) )
                {
                    res[i].sasa = atoi(buffer+35);

```

319

```

        i++;
    }
    else
        printf("Inconsistency between pdb and dssp file at residue
5  %c%d\n",res[i].ltr, res[i].number);
    }
}
if (i != numofres)
10  printf("Inconsistency between pdb and dssp file: wrong # of residues (%d) in
    pdb, (%d) in dssp\n", numofres, i);

    return i;
}
15

void writedatafile(char *filename)
{
    20  int i;
        FILE *outfile;

    if (outfile = fopen(filename, "w"))
25  {
        fprintf(outfile, "# seq  pdb AA  epitopes\n");
        fprintf(outfile, "#      seq          \n");
        for (i=0; i<numofres; i++)
            fprintf(outfile, "%4d %4d  %c %4d\n", i+1 , res[i].number, res[i].ltr,
30  res[i].member_of_epitopes);

        fclose(outfile);
    }

35 }

/* ----- ANALYSIS FUNCTIONS ----- */

int addchild(int parent, int residue, char level)
40 {
    if (numofepires == MAXEPITOPERES)
    {
        printf("Sorry, program constant MAXEPITOPERES exceeded, increase and recompile
        program\n");
45  exit (0);
    }

    epires[numofepires].parent = parent; /* should be -1 for the top level */
    epires[numofepires].residue = residue; /* should be -1 for a gap */
50  epires[numofepires].level = level;

    numofepires++;

    /*
55  if (numofepires % 10 == 0)
        printf ("Added %d epires\n",numofepires);
    */

    return numofepires;
60 }

float sqdist(int i, int j)
{
    /* returns the square of the distance between the coordinates for residues i and j
65 */

```

320

```

    return (res[i].x-res[j].x)*(res[i].x-res[j].x)+(res[i].y-res[j].y)*(res[i].y-
res[j].y)+(res[i].z-res[j].z)*(res[i].z-res[j].z);
}

5 void findepitopes(void) /* This is the core algorithm */
{
    int i, j, k, nogapanchestor;

10 /* --- Find parents --- */

    for(i=0; i<numofres; i++)
        if (res[i].sasa >= minsasa && strchr(consensus[0],res[i].ltr))
15         addchild(-1,i,0);

    /* ---- do 'consensuslength-1' number of child cycles ----- */

20 for (i=1; i<consensuslength; i++)
    for (j=numofepires-1; j>=0 && epires[j].level == i-1; j--)
    {
        if (strchr(consensus[i],'-')) /* is a gap allowed at this position in the
25 consensus ? */
            addchild(j,-1,i);

        if (epires[j].residue == -1) /* this a gap, so use distance to parents (or
older ancestor) instead */
30     {

        /* the following line is for handling multiple gaps after each other */
        for (nogapanchestor = epires[j].parent; epires[nogapanchestor].residue == -
1; nogapanchestor = epires[nogapanchestor].parent);
35
        for(k=0; k<numofres; k++)
        /* if (res[k].sasa >= minsasa && strchr(consensus[i],res[k].ltr) && k !=
epires[epires[j].parent].residue && sqdist(k,epires[epires[j].parent].residue) <=
sqmindist) */
40         if (res[k].sasa >= minsasa && strchr(consensus[i],res[k].ltr) && k !=
epires[nogapanchestor].residue && sqdist(k,epires[nogapanchestor].residue) <= sqmin-
dist)
            addchild(j,k,i);
        }
45     else
    {
        for(k=0; k<numofres; k++)
            if (res[k].sasa >= minsasa && strchr(consensus[i],res[k].ltr) && k !=
50 epires[j].residue && sqdist(k,epires[j].residue) <= sqmindist)
                addchild(j,k,i);
        }
    }

55 longestepitope = epires[numofepires-1].level+1;
}

60 int cmp(const void *a, const void *b)
{
    struct epitope *aa = (struct epitope *)a;
    struct epitope *bb = (struct epitope *)b;

65 if (aa->sasa < bb->sasa)
    return 1;

```

321

```

    else if (aa->sasa == bb->sasa)
        return 0;
    else
        return -1;
5 }

void processepitopes(void) /* Go through the epitopes, remove copies, nonsense se-
quences etc. */
10 {
    int i, j, k, l, n, thisepinnumbers[MAXCONSENSUS], processed=0;
    char thisepi[255], tmp[50];
    char discarded, toobig, onepresent, allpresent;
    float maxsqdist;
15
    for (i=numofepires-1; i>=0 && epires[i].level == epires[numofepires-1].level; i-
-)
    {
20        discarded = 0; toobig = 0;
        strcpy(thisepi, "");
        j = i;
        n = 0;
        maxsqdist = 0;
25        do {
            thisepinnumbers[n++] = epires[j].residue;

            if (epires[j].residue == -1) /* its a gap */
                sprintf(tmp, "---, ");
30            else
                sprintf(tmp, "%c%d, ", res[epires[j].residue].ltr,
res[epires[j].residue].number);

            if (strstr(thisepi, tmp) && epires[j].residue != -1) /* only gaps can be
35 present twice! */
                discarded = 1;
            else
                strcat(thisepi, tmp);

40            j=epires[j].parent;
        } while (j != -1);

        for (k=0; k <= epires[numofepires-1].level; k++)
            for (l=k+1; l <= epires[numofepires-1].level; l++)
45            if (thisepinnumbers[k] != -1 && thisepinnumbers[l] != -1) /* if there are
no gaps involved */
                maxsqdist = max(maxsqdist, sqdist(thisepinnumbers[k], thisepinnumbers[l])
            );

50        if (maxsqdist > sqmaxsize)
            toobig = 1;

        if (toobig)
55            discarded = 1;

        if (!discarded) /* put the found epitopes into the epitope list */
        {
60            sprintf(epi[numofepitopes].epi, "%s\n", thisepi);
            epi[numofepitopes].sasa = 0;
            epi[numofepitopes].gaps = 0;
            epi[numofepitopes].residues = 0;
            epi[numofepitopes].size = sqrt(maxsqdist);
65            for (j = 0; j < n; j++) /* loop over the residues in this epitope */
            {

```


322

```

        epi[numofepitopes].res[j] = thisepinnumbers[j];    /* copy the residue num-
bers to the epitope list */

        if (thisepinnumbers[j] != -1)                      /* if it is not a gap
5 */
        {
            epi[numofepitopes].sasa += res[thisepinnumbers[j]].sasa;
            epi[numofepitopes].residues++;
        }
10        else
            epi[numofepitopes].gaps++;

        }
        numofepitopes++;
15        if (numofepitopes == MAXEPITOPES)
        {
            printf("MEXEPITOPES exceeded. Increase and recompile program.\n");
            exit(0);
        }
20    }

    }

    /* now indetify epitopes which are a subset of others */
25    for (i=0; i<numofepitopes; i++) /* initialize array */
        epi[i].subset = 0;

    for (i=0; i<numofepitopes; i++)
30    {
        for (j=0; j<numofepitopes; j++)
        {
            if (epi[i].residues > epi[j].residues)
            {
35                allpresent = 0;
                for (k=0; k<epi[i].residues; k++)
                {
                    if (epi[i].res[k] != -1)
                    {
40                        onepresent = 0;
                        for (l=0; l<epi[j].residues; l++)
                            if (epi[i].res[k] == epi[j].res[l]) /* if the residues are the same
and not gaps */
                                onepresent = 1;
45                        allpresent |= onepresent;
                    }
                }
                if (allpresent)
                {
50                    epi[j].subset = 1;
                    numofsubsets++; */
                }
            }
        }
55    }

    /* now sort the epitopes according to SASA */
60    qsort(&(epi[0]), numofepitopes, sizeof(struct epitope), &cmp);

    /* counts the ones that are subsets of others */

    for (i=0; i<numofepitopes; i++)
65    {
        if (epi[i].subset == 1)
            numofsubsets++;
    }

```

```

    /* now count how many epitopes each residue is a member of,
       considering only non-redundant epitopes, and the number of epitopes wanted */
5   for (i=0; i < numofepitopes && processed < maxepi; i++)
       if (epi[i].subset == 0) /* count only if the epitope is not a subset of an-
other */
       {
           processed++;
10          for (j=0; j < epi[i].residues; j++)
               (res[epi[i].res[j]].member_of_epitopes)++; /* add the counter for epi-
topes for the residues */
       }
15 }

void printepitopes(void)
{
20   int i, processed = 0;

       for (i=0; i < numofepitopes && processed < maxepi; i++)
           if (epi[i].subset == 0)
           {
25             printf("SAS: %3d, Size %5.2f: %s", epi[i].sasa, epi[i].size, epi[i].epi);
               processed++;
           }
       }

30 void usage (void)
{
    fprintf(stderr, "USAGE: epitope <epitope template> <filename_template> dist acc
maxsize number minlength\n");
35   fprintf(stderr, "\n");
    fprintf(stderr, "filenames <filename_template>.pdb and <file-
name_template>.dssp\n");
    fprintf(stderr, "           must be present.\n");
    fprintf(stderr, "dist is the maximum distance between adjacent residues in epi-
40 tope.\n");
    fprintf(stderr, "acc is minimum surface accessible area in square angstroms.\n");
    fprintf(stderr, "maxsize is the maximum distance between any two residues in the
epitope.\n");
    fprintf(stderr, "number is the maximum number of non-redundant epitopes to consider
45 (0=all)\n");
    fprintf(stderr, "minlength is the minimum length of the epitope seqs (in frac-
tions\n");
    fprintf(stderr, "   of the consensus sequence length).\n");
    fprintf(stderr, "A file <filename_template>.dat containing the number of epi-
50 topes\n");
    fprintf(stderr, "each residue participates in is written.\n");
    fprintf(stderr, "\n");

    exit(0);
55 }

int main (int argc, char **arg)
60 {
    int i;
    char pdbfile[256], dsspfile[256], datfile[256];

    if (argc != 8)
65     usage();

    readconsensus(arg[1]);

```

324

```

printf ("Epitope consensus sequence read from %s\n",arg[1]);
printf ("-----\n");
for (i = 0; i < consensuslength; i++)
5   printf("%s\n",consensus[i]);
printf("\n");

strcpy(pdbfile,arg[2]);
strcat(pdbfile,".pdb");
10  strcpy(dsspfile,arg[2]);
strcat(dsspfile,".dssp");

strcpy(datfile,arg[2]);
15  strcat(datfile,".dat");

readpdbCA(pdbfile);

printf ("Sequence read from %s\n",pdbfile);
20  printf ("-----\n");
for (i = 0; i < numofres; i++)
{
    printf("%c",res[i].ltr);
    if (!(i+1)%70)
25  printf("\n");
}

printf("\n\n");

30  readssp(dsspfile);

mindist = atof(arg[3]);
minsasa = atoi(arg[4]);
maxsize = atof(arg[5]);
35  maxepi = atoi(arg[6]);
if (maxepi == 0)
    maxepi = INT_MAX;
minlength = atof(arg[7]); /* minimum length of epitope sequence (in fractions
of the consensus length) */
40  sqmindist = mindist*mindist;
sqmaxsize = maxsize*maxsize;

minlength_residues = (float) ceil(minlength*consensuslength);
45  findepitopes();

if (longestepitope >= minlength_residues)
    processepitopes();
50  printf ("Parameters and internal numbers\n");
printf ("-----\n");
printf ("Program revision date           : %s\n", REVISIONDATE);
printf ("Consensus sequence length          : %d\n", consensuslength);
55  printf ("Minimum epitope seq length threshold : %.2f (%d residues)\n",
minlength, minlength_residues);
printf ("Longest epitope sequence found       : %d\n", longestepitope);
printf ("Number of residues in PDB file       : %d\n", numofres);
printf ("Distance threshold value (angstroms) : %.1f\n", mindist);
60  printf ("Minimum surface accessible area of each res : %d\n", minsasa);
printf ("Maximum epitope size                  : %.1f\n", maxsize);
printf ("Number of nodes in epitope tree      : %d\n", numofepires);
printf ("Total number of epitopes....         : %d\n", numofepitopes);
printf ("....of which are subsets of others   : %d\n", numofsubsets);
65  printf ("Max number of non-redundant epitopes  : %d\n", maxepi);
printf ("\n");

```

325

```
    printf ("Epitopes found\n");
    printf ("-----\n");

    if (longestepitope >= minlength_residues)
5      printepitopes();

    writedatafile(datfile);

/*
10   for (i = 0; i < numofepires; i++)
        printf("|%4d %4d %4d %4d  ", i, epires[i].level, epires[i].residue,
        epires[i].parent);

*/
15   return 0;
}
```

Appendix B**The wrapper (Python) (epitope5.cgi)**

```

5      #!/z/vaks/bin/python
      #
      # Automatic epitope mapping
      #
10     import cgi, os, time, commands, string, sys

      FormFile = "epitope.html"
      scriptdir = "/z/edhome/epf/public_html/epitope/"
15     epitopepath = "/z/edhome/epf/epitope/epitope3"
      dssppath = "/z/vaks/bin/dssp"
      gnuplotpath = "/z/edhome/epf/gnuplot-3.7/gnuplot"
      zippath = "/usr/freeware/bin/zip"
      unzippath = "/usr/freeware/bin/unzip"
20     timestamp = str(int(time.time()))

      liball = range(1,53)
      libigg = [3,4,7,11,14,16,17,30,31,32,34,35,38,39,41,42,43,47,48,49,50,51,52]
25     libige =
        [1,2,5,6,8,9,10,12,13,15,18,19,20,21,22,23,24,25,26,27,28,29,33,36,37,40,44,45,46]

30     # ----- the page startes here -----

      print "Content-type: text/html\n\n"    # HTML is following

      print '<html>\n'
35     print '<head>\n'

      print '<title>Automatic epitope mapping</title>\n'
      print '</head>\n'
      print '\n'
40     # ----- check for lock file

      if os.path.isfile("epitope.lock"):
          print 'Sorry - lock file exists. This means that automatic epitope mapping is al-
45 ready in use,'
          print 'or that an error has occured.<BR>'
          print "If you are absolutely sure that no one are using automatic epitope mapping,
you can"
          print "press the button below. <BR>"
50     print "If you are not sure, just press 'back' in your browser now."

          print '<BR><BR>'
          print '<form METHOD=GET AC-
TION="http://vaks.novo.dk/-epf/epitope/epitope_remove.lock.cgi"><input type="submit"
55 name="SUBMIT_BUTTON" value="Remove lock file"></form>'

          sys.exit(0)

60     # ----- create lock file -----

      os.system ("touch epitope.lock")

65     # ----- Clean up directory -----
      # --- (delete everything but md_analysis.cgi and md_analysis.html) ---

```

```

#commands.getoutput("ls -l | awk '$9 !~ /^epitope/ {print \"rm\",$9}' >cleanup.sh")
#commands.getoutput(" " + scriptdir + "cleanup.sh")
5
#if os.path.isfile("cleanup.sh"):
# os.remove ("cleanup.sh")

commands.getoutput ("rm *.png")
10 commands.getoutput ("rm *.dat.txt")
commands.getoutput ("rm *.out.txt")

# remove any subdirs

15 commands.getoutput ("find . -type d -name '???' -exec rm -rf {} \;")

# ----- the page continues here -----

form = cgi.FieldStorage()
20

infile = form["pdbfile"].value

namebase = form["pdbfile"].filename
namebasenum = string.rfind(namebase, '\\')
25 if namebasenum < -1:
    namebasenum = 0

namelist = string.split(namebase[namebasenum+1:], '.')
30
pdbname = namelist[0] + '.pdb'
dsspname = namelist[0] + '.dssp'
datname = namelist[0] + '.dat'
dattxtname = namelist[0] + '.dat.txt'
35 zipname = namelist[0] + '.zip'
inzipname = 'submitted.zip'

consensusname = namelist[0] + '.cons'
epiname = namelist[0] + '.out.txt'
40
minsasa = form["minsasa"].value
mindist = form["mindist"].value
maxsize = form["maxsize"].value
consensus = form["consensus"].value
45 threshold = form["threshold"].value
number = form["number"].value
minlength = form["minlength"].value
plotmode = form["plot_mode"].value
operatemode = form["operate_mode"].value
50 if (operatemode[0:7] == "library"):
    operatemode = "library"

    if (form["operate_mode"].value == "library_all"):
        lib = liball
55 elif (form["operate_mode"].value == "library_igg"):
        lib = libigg
    elif (form["operate_mode"].value == "library_ige"):
        lib = libige
    if (operatemode == "library"):
60 libsize = len(lib)

    if (string.upper(namelist[1]) == 'PDB'):
        inputtype = 'PDB'
    if (string.upper(namelist[1]) == 'ZIP'):
65 inputtype = 'ZIP'

# ----- write submitted file

```

```

    if (inputtype == 'PDB'):
        f=open(pdbname, "w")
    if (inputtype == 'ZIP'):
5      f=open(inzipname, "w")
        f.write(infile)
        f.close()

    # ----- If the submitted file is a zip-file, extract it and make a list of the en-
10   tries -----

    if (inputtype == 'ZIP'):
        pdbfiles = string.split(commands.getoutput(unzippath+" -l "+inzipname+" | awk '{ if
        (NR > 3 && NF == 4) print $4}'"))
15      numofpdbfiles = len(pdbfiles)
        commands.getoutput(unzippath+" -j "+inzipname)

        # ----- make directories and move the zipfiles there -----

20      for i in pdbfiles:
            dirname = i[0:-4]
            commands.getoutput("rm -rf "+dirname)
            os.mkdir(dirname)
            os.rename(i,dirname+"/"+i)
25      else:
            pdbfiles = [pdbname]

30      # -----

        if (operatemode == "single"):
            f=open(consensusname, "w")
            f.write(consensus)
35      f.close()

        print '<CENTER>\n'
        if form.has_key("pagetitle"):
40      print '<H1>'+form["pagetitle"].value+'</H1>\n'

        print time.ctime(time.time())+'<BR><BR>\n'

        if (operatemode == "single"):
45      print '<BR><H2>You should print or save this page!</H2>\n'
            print 'The results shown on this page are not stored anywhere else.\n\n'

        if (operatemode == "library"):
            if (inputtype == 'ZIP'):
50      print '<H2><A HREF="collected.zip">Download</A> your results!</H2>\n'
                if (inputtype == 'PDB'):
                    print '<H2><A HREF="'+zipname+'">Download</A> your results!</H2>\n'
                    print 'Downloading is strongly recommended! The results are shown on this page and
                    included\n'
55      print 'in this archive. They are not stored anywhere else.<BR><BR>\n'

            print 'Filename given by you:<BR>\n'
            print '<B>'+form["pdbfile"].filename+'</B>\n'

60

        # ----- run the program -----

        #if (inputtype == 'ZIP'):
65      if (1 == 1):

            for currentpdbname in pdbfiles:

```

```

# ----- the naming stuff - identical to that at the top of the file ---

namebase = currentpdbname
5 namebasenum = string.rfind(namebase, '\\')
  if namebasenum < -1:
    namebasenum = 0

namelist = string.split(namebase[namebasenum+1:], '.')
10
  if (inputtype == 'PDB'):
    nameroot = namelist[0]
  if (inputtype == 'ZIP'):
    nameroot = namelist[0]
15 #   nameroot = currentpdbname[0:-4]+"/"+namelist[0]

  pdbname = nameroot+'.pdb'
  dsspname = nameroot+'.dssp'
  datname = nameroot+'.dat'
20  dattxtname = nameroot+'.dat.txt'
  zipname = nameroot+'.zip'

  epiname = nameroot+'.out.txt'

25
# ----- here comes the treatment of the individual structures -----

  if (inputtype == 'ZIP'):
    os.chdir(currentpdbname[0:-4])
30
  if (operatemode == "single"):

    # add extra newlines to the consensus file

35    commands.getoutput("echo \\n\\n\\n >> "+consensusname)

    commands.getoutput(dssppath+" "+pdbname+" "+dsspname)

    if (inputtype == 'ZIP'):
40      commands.getoutput(epitopepath+" ../"+consensusname+" "+namelist[0]+"
"+mindist+" "+minsasa+" "+maxsize+" "+number+" "+minlength+" > "+epiname)
    else:
      commands.getoutput(epitopepath+" "+consensusname+" "+namelist[0]+"
"+mindist+" "+minsasa+" "+maxsize+" "+number+" "+minlength+" > "+epiname)
45      commands.getoutput("mv "+datname+" "+dattxtname)

  if (operatemode == "library"):

50    commands.getoutput(dssppath+" "+pdbname+" "+dsspname)
  #   for i in range(1,libsize+1):
    for i in lib:
      if (inputtype == 'ZIP'):
        commands.getoutput(epitopepath+" ../"+string.zfill(str(i),3)+".epi
55 "+namelist[0]+" "+mindist+" "+minsasa+" "+maxsize+" "+number+" "+minlength+" >
"+string.zfill(str(i),3)+".out.txt")
      else:
        commands.getoutput(epitopepath+" "+string.zfill(str(i),3)+".epi
"+namelist[0]+" "+mindist+" "+minsasa+" "+maxsize+" "+number+" "+minlength+" >
60 "+string.zfill(str(i),3)+".out.txt")
        commands.getoutput("mv "+datname+" "+string.zfill(str(i),3)+".dat.txt")
        residues = int(commands.getoutput("grep -v '#'
"+string.zfill(str(lib[0]),3)+".dat.txt | wc | awk '{print $1}'"))
        commands.getoutput("rm sum.dat.txt")
65      for i in range(1,residues+1):
        grepstr = "^"+string.rjust(str(i),4)

```


330

```

        commands.getoutput("grep '"+grepstr+"' *.dat.txt | awk 'BEGIN{sum=0}{sum+=$5;
res=$2; pdbres=$3; AA=$4} END{print res, pdbres, AA,sum}' >> sum.dat.txt")
        commands.getoutput("rm "+datname)

5      # ----- collect generated files -----

        if (inputtype == 'PDB'):
            commands.getoutput("rm "+zipname)
            commands.getoutput(zippath+" "+zipname+" *.out.txt *.dat.txt")
10

        # ----- if in library mode, create and show the sum graph -----

15
        if (operatemode == "library"):
            timestamp = str(int(time.time()))

            f=open("epitope.gnp", "w")
            if (plotmode == "sequential"):
20                f.write('set xlabel "Residue number (sequential)"\n')
            else:
                f.write('set xlabel "Residue number (PDB)"\n')
                f.write('set ylabel "Epitopes"\n')
25                f.write('set title '"+currentpdbname[0:-4]+'"\n')
                f.write('set size ratio 0.3 1, 0.5\n')
                f.write('set term png small color\n')
                f.write('set out "epi'+timestamp+'.png"\n')
                if (plotmode == "sequential"):
30                    f.write('plot "sum.dat.txt" using 1:4 title "Number of epitopes" with steps
1, '+threshold+' title "Threshold" with lines 3\n')
                else:
                    f.write('plot "sum.dat.txt" using 2:4 title "Number of epitopes" with steps
1, '+threshold+' title "Threshold" with lines 3\n')
35                f.close()

            commands.getoutput(gnuplotpath+" epitope.gnp")

            print '<H1>Epitope frequency sums for each residue</H1><BR>\n'

40
            if (form["operate_mode"].value == "library_all"):
                print '<H2>Library of '+str(libsize)+' epitopes (IgG+IgE)</H2>'
            elif (form["operate_mode"].value == "library_igg"):
                print '<H2>Library of '+str(libsize)+' epitopes (IgG)</H2>'
45            elif (form["operate_mode"].value == "library_ige"):
                print '<H2>Library of '+str(libsize)+' epitopes (IgE)</H2>'

            if (inputtype == 'PDB'):
                print '<BR><BR><IMG SRC="epi'+timestamp+'.png"><BR><BR>\n'
50                print '<A HREF="sum.dat.txt">View the frequency sums table data</A><BR>\n'
                print '<A HREF="'+zipname+">Download</A> a zip file with all results from
the individual epitopes.<BR>\n'
                print '</CENTER>\n'

55            if (inputtype == 'ZIP'):
                print '<BR><BR><IMG SRC="'+currentpdbname[0:-
4]+'epi'+timestamp+'.png"><BR><BR>\n'
                print '<A HREF="'+currentpdbname[0:-4]+'/sum.dat.txt">View the frequency sums
table data</A><BR>\n'
60

        # ----- now make gnuplot graphs and data lists for individual epitopes -----
        ----

65      # --- so far this goes only for the "single" operating mode -----

```

331

```

if (operatemode == "single"):
    timestamp = str(int(time.time()))

    # Create gnuplot control file
5
    f=open("epitope.gnp", "w")
    if (plotmode == "sequential"):
        f.write('set xlabel "Residue number (sequential)"\n')
    else:
10
        f.write('set xlabel "Residue number (PDB)"\n')
        f.write('set ylabel "Epitopes"\n')
        f.write('set size ratio 0.3 1, 0.5\n')
        f.write('set term png small color\n')
        f.write('set out "epi'+timestamp+'.png"\n')
15
        if (plotmode == "sequential"):
            f.write('plot "' + dattxtname + '" using 1:4 title "Number of epitopes" with
steps 1, '+threshold+' title "Threshold" with lines 3\n')
        else:
            f.write('plot "' + dattxtname + '" using 2:4 title "Number of epitopes" with
20 steps 1, '+threshold+' title "Threshold" with lines 3\n')
        f.close()

    commands.getoutput(gnuplotpath+" epitope.gnp")

25
    if (inputtype == 'ZIP'):
        print '<BR><BR><IMG SRC="'+currentpdbname[0:-
4]+'/epi'+timestamp+'.png"><BR><BR>\n'
        print '<A HREF="'+currentpdbname[0:-4]+'/' + dattxtname + '">View the table da-
ta</A><BR>\n'
30
    else:
        print '<BR><BR><IMG SRC="epi'+timestamp+'.png"><BR><BR>\n'
        print '<A HREF="'+dattxtname+'">View the table data</A><BR>\n'
        print '</CENTER>\n'

35
    # ----- print the table -----

    print '<PRE>'
    f=open(epiname, "r")
40
    line = f.readline()

    while line != "":
        line = string.replace(line, '\n', '')
        print line
45
        line = f.readline()

    f.close()
    print '</PRE><BR><BR><BR>'

50
    # -----

    if (inputtype == 'ZIP'):
        os.chdir("../")
55

    # ----- for ZIP-mode (library only): count number of epitopes found from each
    lib consensus ----

60

    if (inputtype == 'ZIP' and operatemode == "library"):

        numofepitopes = []

65
        f=open("epitopecount.txt", "w")
        f.write(string.ljust("PDB file", 20))

```

```

    for i in lib:
        f.write(string.rjust(str(i),6))
    f.write('\n')

5   for j in range(len(pdbfiles)):
        currentpdbname = pdbfiles[j]
        f.write(string.ljust(currentpdbname[0:20],20))
        for idx in range(len(lib)):
            i = lib[idx]
10        filename = currentpdbname[0:-4]+"/"+string.zfill(str(i),3)+".out.txt"
            numofepitopes.append(0)
            tmp = commands.getoutput("grep 'Total number of epitopes' "+filename+" | awk
            '{print $6}'")
            if (tmp != ""):
15                numofepitopes[j*len(pdbfiles)+idx] = int(tmp)
                numofepitopes[j*len(pdbfiles)+idx] = numofepitopes[j*len(pdbfiles)+idx]-
                int(commands.getoutput("grep 'of which are subsets' "+filename+" | awk '{print
                $8}'"))
            else:
20                numofepitopes[j*len(pdbfiles)+idx] = 0
                f.write(string.rjust(str(numofepitopes[j*len(pdbfiles)+idx]),6))
                f.write('\n')

        f.close()

25 # ----- for ZIP-mode: Collect all dirs and files -----

    if (inputtype == 'ZIP'):
        commands.getoutput("rm collected.zip")
30    for currentpdbname in pdbfiles:
        commands.getoutput(zippath+" -r -u collected.zip "+currentpdbname[0:-4])
        if (operatemode == "library"):
            commands.getoutput(zippath+" -u collected.zip epitopecount.txt")

35 # ---- Last lines ----

    print '</body>\n'
    print '</html>\n'

40 # ---- remove lock file -----

    os.remove ("epitope.lock")

45 # ----- remove temporary files -----

    #if (inputtype == 'ZIP'):
    #    for currentpdbname in pdbfiles:
    #        commands.getoutput("rm -rf "+currentpdbname[0:-4])
50    commands.getoutput ("rm "+pdbname)
    commands.getoutput ("rm "+dsspname)
    commands.getoutput ("rm "+consensusname)
    commands.getoutput ("rm "+epiname)
55

```

Appendix C

The HTML input form (epitope5.html)

[illegible]

```

<TR><TD>*      </TD><TD></TD><TD> (All residues allowed, but there must be a resi-  
due)</TD><TR>  
<TR><TD>?      </TD><TD></TD><TD> (All or missing residue allowed)</TD><TR>  
<TR><TD>DE      </TD><TD></TD><TD> (Asp or Glu allowed)</TD><TR>  
5 </TABLE>  
<BR>  
*, ? or - in first or last position is allowed but obsolete.  
(- in first position is ignored.)  
  
10 </TD></TR>  
</TABLE>  
<BR><HR WIDTH=80%><BR>  
  
<TABLE>  
15 <TR>  
<TD>Maximum distance between adjacent residues </TD><TD><INPUT type=text na-  
me="mindist" size="5" maxlength="8" value = "10"></TD>  
</TR>  
<TR>  
20 <TD>Minimum solvent accessible surface area for each residue</TD><TD><INPUT type=text  
name="minsasa" size="5" maxlength="8" value = "5"></TD>  
</TR>  
<TR>  
<TD>Maximum epitope size (max distance between any two residues in epi-  
tope)</TD><TD><INPUT type=text name="maxsize" size="5" maxlength="8" value =  
25 "25"></TD>  
</TR>  
<TR>  
<TD>Maximum number of non-redundant epitopes to include (0 = all)</TD><TD><INPUT  
30 type=text name="number" size="5" maxlength="8" value = "0"></TD>  
</TR>  
<TD>Minimum epitope sequence length (in fractions of consensus length)</TD><TD><INPUT  
type=text name="minlength" size="5" maxlength="8" value = "0.80"></TD>  
</TR>  
35 </TABLE>  
<BR><HR WIDTH=80%><BR>  
<H2>Graph</H2>  
  
<INPUT TYPE=RADIO NAME="plot_mode" VALUE="sequential" CHECKED>  
40 &nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&Use sequential numbering of residues.<BR>  
<INPUT TYPE=RADIO NAME="plot_mode" VALUE="pdb">  
&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&Use PDB numbering of residues. (Will sometimes produce funny re-  
sults.)<BR>  
Threshold value &nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&<INPUT type=text name="threshold" size="5" max-  
45 length="8" value = "2"><BR>  
  
<BR><HR WIDTH=80%><BR>  
  
<input type="submit" name="SUBMIT_BUTTON" width=100 value="Find epitopes"></form>  
50 <form METHOD=GET ACTION="./epitope.html"><input type="submit" name="SUBMIT_BUTTON"  
width=100 value="Reset form">  
</form>  
<HR WIDTH=80%><BR>  
  
55 <BR>  
<CENTER>  
Comments and bug reports to <A HREF="mailto:epf@novo.dk">epf</A>. .  
<BR><BR>  
<IMG SRC="./epitope_nz.gif">  
60 </CENTER>  
</body>  
</html>
```

Appendix D 3D Structure of Esperase

	ATOM	1	N	GLN	A	2	24.343	43.495	26.356	1.00	26.00	7
5	ATOM	2	NE2	GLN	A	2	25.686	39.582	30.163	1.00	20.88	7
	ATOM	3	OE1	GLN	A	2	23.497	39.261	29.938	1.00	23.07	8
	ATOM	4	CD	GLN	A	2	24.448	40.036	29.883	1.00	23.09	6
	ATOM	5	CG	GLN	A	2	24.420	41.507	29.607	1.00	23.93	6
	ATOM	6	CB	GLN	A	2	24.309	41.801	28.125	1.00	23.06	6
10	ATOM	7	CA	GLN	A	2	23.999	43.235	27.778	1.00	25.53	6
	ATOM	8	C	GLN	A	2	24.957	44.096	28.566	1.00	28.66	6
	ATOM	9	O	GLN	A	2	26.126	44.049	28.148	1.00	31.97	8
	ATOM	10	N	THR	A	3	24.538	44.857	29.557	1.00	25.20	7
	ATOM	11	CG2	THR	A	3	24.948	47.593	29.045	1.00	32.60	6
15	ATOM	12	OG1	THR	A	3	23.634	46.905	30.890	1.00	33.76	8
	ATOM	13	CB	THR	A	3	24.979	47.085	30.464	1.00	26.52	6
	ATOM	14	CA	THR	A	3	25.508	45.643	30.316	1.00	24.44	6
	ATOM	15	C	THR	A	3	25.551	45.035	31.717	1.00	23.97	6
	ATOM	16	O	THR	A	3	24.566	44.377	32.092	1.00	27.28	8
20	ATOM	17	N	VAL	A	4	26.585	45.366	32.449	1.00	24.67	7
	ATOM	18	CG2	VAL	A	4	28.377	43.274	33.058	1.00	22.99	6
	ATOM	19	CG1	VAL	A	4	28.147	43.784	35.492	1.00	22.90	6
	ATOM	20	CB	VAL	A	4	28.128	44.351	34.069	1.00	24.23	6
	ATOM	21	CA	VAL	A	4	26.694	44.897	33.837	1.00	24.05	6
25	ATOM	22	C	VAL	A	4	26.445	46.114	34.776	1.00	22.35	6
	ATOM	23	O	VAL	A	4	27.323	47.015	34.816	1.00	24.67	8
	ATOM	24	N	PRO	A	5	25.365	46.082	35.507	1.00	21.36	7
	ATOM	25	CD	PRO	A	5	24.284	45.039	35.492	1.00	16.33	6
	ATOM	26	CG	PRO	A	5	23.100	45.761	36.119	1.00	19.38	6
30	ATOM	27	CB	PRO	A	5	23.741	46.724	37.115	1.00	17.69	6
	ATOM	28	CA	PRO	A	5	25.049	47.159	36.454	1.00	17.81	6
	ATOM	29	C	PRO	A	5	26.231	47.367	37.382	1.00	24.17	6
	ATOM	30	O	PRO	A	5	26.903	46.375	37.763	1.00	19.11	8
	ATOM	31	N	TRP	A	6	26.505	48.602	37.832	1.00	21.75	7
35	ATOM	32	CD2	TRP	A	6	26.928	50.889	41.509	1.00	18.89	6
	ATOM	33	CE3	TRP	A	6	27.995	50.522	42.349	1.00	19.68	6
	ATOM	34	CZ3	TRP	A	6	27.789	50.639	43.721	1.00	18.65	6
	ATOM	35	CH2	TRP	A	6	26.582	51.111	44.306	1.00	18.90	6
	ATOM	36	CZ2	TRP	A	6	25.524	51.469	43.465	1.00	18.51	6
40	ATOM	37	CE2	TRP	A	6	25.705	51.348	42.088	1.00	24.32	6
	ATOM	38	NE1	TRP	A	6	24.852	51.593	41.020	1.00	22.59	7
	ATOM	39	CD1	TRP	A	6	25.420	51.300	39.828	1.00	14.24	6
	ATOM	40	CG	TRP	A	6	26.698	50.865	40.074	1.00	17.07	6
	ATOM	41	CB	TRP	A	6	27.702	50.382	39.095	1.00	19.96	6
45	ATOM	42	CA	TRP	A	6	27.668	48.899	38.677	1.00	19.10	6
	ATOM	43	C	TRP	A	6	27.699	48.015	39.926	1.00	20.24	6
	ATOM	44	O	TRP	A	6	28.865	47.719	40.230	1.00	19.68	8
	ATOM	45	N	GLY	A	7	26.553	47.779	40.554	1.00	19.54	7
	ATOM	46	CA	GLY	A	7	26.573	47.016	41.827	1.00	15.44	6
50	ATOM	47	C	GLY	A	7	27.075	45.596	41.634	1.00	21.44	6
	ATOM	48	O	GLY	A	7	27.733	45.067	42.534	1.00	20.88	8
	ATOM	49	N	ILE	A	8	26.862	44.983	40.482	1.00	19.17	7
	ATOM	50	CD1	ILE	A	8	24.548	42.180	39.852	1.00	19.08	6
	ATOM	51	CG1	ILE	A	8	25.219	43.020	38.790	1.00	17.53	6
55	ATOM	52	CB	ILE	A	8	26.746	43.093	38.871	1.00	23.00	6
	ATOM	53	CG2	ILE	A	8	27.338	41.799	38.350	1.00	22.68	6
	ATOM	54	CA	ILE	A	8	27.325	43.598	40.192	1.00	23.07	6
	ATOM	55	C	ILE	A	8	28.853	43.585	40.232	1.00	22.71	6
	ATOM	56	O	ILE	A	8	29.462	42.674	40.821	1.00	21.85	8
60	ATOM	57	N	SER	A	9	29.527	44.534	39.631	1.00	19.30	7

336

	ATOM	58	OG	SER	A	9	31.089	45.298	37.438	1.00	28.25	8
	ATOM	59	CB	SER	A	9	31.514	45.590	38.718	1.00	24.45	6
	ATOM	60	CA	SER	A	9	30.986	44.532	39.663	1.00	18.00	6
	ATOM	61	C	SER	A	9	31.431	45.071	41.000	1.00	18.16	6
5	ATOM	62	O	SER	A	9	32.543	44.676	41.351	1.00	21.78	8
	ATOM	63	N	PHE	A	10	30.702	45.961	41.617	1.00	17.83	7
	ATOM	64	CD2	PHE	A	10	31.780	49.344	44.181	1.00	23.83	6
	ATOM	65	CE2	PHE	A	10	32.100	50.259	45.170	1.00	27.32	6
	ATOM	66	CZ	PHE	A	10	31.514	50.266	46.431	1.00	21.18	6
10	ATOM	67	CE1	PHE	A	10	30.563	49.309	46.768	1.00	29.76	6
	ATOM	68	CD1	PHE	A	10	30.188	48.429	45.759	1.00	23.23	6
	ATOM	69	CG	PHE	A	10	30.778	48.438	44.521	1.00	18.74	6
	ATOM	70	CB	PHE	A	10	30.285	47.522	43.455	1.00	17.70	6
	ATOM	71	CA	PHE	A	10	31.270	46.528	42.864	1.00	20.00	6
15	ATOM	72	C	PHE	A	10	31.457	45.396	43.870	1.00	22.92	6
	ATOM	73	O	PHE	A	10	32.357	45.569	44.723	1.00	24.39	8
	ATOM	74	N	ILE	A	11	30.614	44.376	43.829	1.00	19.21	7
	ATOM	75	CD1	ILE	A	11	27.476	41.276	44.648	1.00	14.26	6
	ATOM	76	CG1	ILE	A	11	28.743	41.954	44.149	1.00	18.25	6
20	ATOM	77	CB	ILE	A	11	29.500	42.669	45.229	1.00	23.27	6
	ATOM	78	CG2	ILE	A	11	28.762	43.839	45.866	1.00	21.09	6
	ATOM	79	CA	ILE	A	11	30.789	43.259	44.739	1.00	20.52	6
	ATOM	80	C	ILE	A	11	31.715	42.170	44.172	1.00	21.46	6
	ATOM	81	O	ILE	A	11	31.783	41.155	44.840	1.00	20.99	8
25	ATOM	82	N	ASN	A	12	32.378	42.329	43.056	1.00	21.03	7
	ATOM	83	ND2	ASN	A	12	35.345	43.095	44.578	1.00	30.69	7
	ATOM	84	OD1	ASN	A	12	36.135	42.268	42.569	1.00	35.13	8
	ATOM	85	CG	ASN	A	12	35.390	42.276	43.541	1.00	25.00	6
	ATOM	86	CB	ASN	A	12	34.450	41.092	43.449	1.00	21.03	6
30	ATOM	87	CA	ASN	A	12	33.340	41.412	42.463	1.00	23.98	6
	ATOM	88	C	ASN	A	12	32.735	40.088	41.978	1.00	24.79	6
	ATOM	89	O	ASN	A	12	33.438	39.085	42.118	1.00	23.07	8
	ATOM	90	N	THR	A	13	31.520	40.204	41.505	1.00	20.38	7
	ATOM	91	CG2	THR	A	13	28.654	38.417	39.642	1.00	15.01	6
35	ATOM	92	OG1	THR	A	13	28.704	40.013	41.326	1.00	22.51	8
	ATOM	93	CB	THR	A	13	29.488	39.474	40.308	1.00	19.67	6
	ATOM	94	CA	THR	A	13	30.810	39.083	40.956	1.00	20.28	6
	ATOM	95	C	THR	A	13	31.671	38.384	39.892	1.00	21.19	6
	ATOM	96	O	THR	A	13	31.605	37.158	39.791	1.00	23.59	8
40	ATOM	97	N	GLN	A	14	32.334	39.049	39.028	1.00	20.22	7
	ATOM	98	NE2	GLN	A	14	32.431	41.889	38.600	1.00	33.33	7
	ATOM	99	OE1	GLN	A	14	31.706	42.497	36.548	1.00	50.01	8
	ATOM	100	CD	GLN	A	14	32.245	41.660	37.297	1.00	52.65	6
	ATOM	101	CG	GLN	A	14	32.764	40.430	36.555	1.00	52.84	6
45	ATOM	102	CB	GLN	A	14	33.857	39.542	37.128	1.00	28.62	6
	ATOM	103	CA	GLN	A	14	33.138	38.429	37.955	1.00	32.46	6
	ATOM	104	C	GLN	A	14	34.201	37.476	38.497	1.00	31.89	6
	ATOM	105	O	GLN	A	14	34.509	36.571	37.705	1.00	27.29	8
	ATOM	106	N	GLN	A	15	34.744	37.757	39.679	1.00	23.92	7
50	ATOM	107	NE2	GLN	A	15	38.511	39.924	42.603	1.00	44.05	7
	ATOM	108	OE1	GLN	A	15	37.542	38.314	43.749	1.00	38.30	8
	ATOM	109	CD	GLN	A	15	37.762	38.831	42.664	1.00	40.79	6
	ATOM	110	CG	GLN	A	15	37.188	38.390	41.331	1.00	34.24	6
	ATOM	111	CB	GLN	A	15	36.297	37.200	41.508	1.00	24.39	6
55	ATOM	112	CA	GLN	A	15	35.728	36.783	40.170	1.00	22.62	6
	ATOM	113	C	GLN	A	15	35.042	35.443	40.384	1.00	29.48	6
	ATOM	114	O	GLN	A	15	35.749	34.432	40.285	1.00	31.32	8
	ATOM	115	N	ALA	A	16	33.762	35.385	40.769	1.00	23.78	7
	ATOM	116	CB	ALA	A	16	31.804	34.146	41.761	1.00	18.00	6
60	ATOM	117	CA	ALA	A	16	33.069	34.097	40.925	1.00	21.90	6
	ATOM	118	C	ALA	A	16	32.825	33.561	39.502	1.00	26.74	6

337

	ATOM	119	O	ALA	A	16	32.967	32.352	39.191	1.00	30.41	8
	ATOM	120	N	HIS	A	17	32.281	34.385	38.577	1.00	30.64	7
	ATOM	121	CD2	HIS	A	17	29.257	34.877	38.233	1.00	22.07	6
	ATOM	122	NE2	HIS	A	17	28.016	35.453	38.259	1.00	25.33	7
5	ATOM	123	CE1	HIS	A	17	27.909	36.328	37.220	1.00	20.45	6
	ATOM	124	ND1	HIS	A	17	29.020	36.372	36.515	1.00	24.91	7
	ATOM	125	CG	HIS	A	17	29.849	35.428	37.109	1.00	22.09	6
	ATOM	126	CB	HIS	A	17	31.222	35.150	36.543	1.00	19.27	6
	ATOM	127	CA	HIS	A	17	31.865	33.972	37.219	1.00	19.98	6
10	ATOM	128	C	HIS	A	17	33.073	33.367	36.512	1.00	29.30	6
	ATOM	129	O	HIS	A	17	32.959	32.347	35.823	1.00	27.69	8
	ATOM	130	N	ASN	A	18	34.191	34.028	36.705	1.00	28.18	7
	ATOM	131	ND2	ASN	A	18	36.859	36.788	35.613	1.00	45.93	7
	ATOM	132	OD1	ASN	A	18	35.325	35.559	34.498	1.00	40.29	8
15	ATOM	133	CG	ASN	A	18	36.220	35.663	35.347	1.00	40.01	6
	ATOM	134	CB	ASN	A	18	36.641	34.520	36.270	1.00	30.63	6
	ATOM	135	CA	ASN	A	18	35.432	33.605	36.085	1.00	27.13	6
	ATOM	136	C	ASN	A	18	35.838	32.250	36.577	1.00	35.11	6
	ATOM	137	O	ASN	A	18	36.705	31.803	35.846	1.00	35.07	8
20	ATOM	138	N	ARG	A	19	35.399	31.756	37.675	1.00	32.73	7
	ATOM	139	NH2	ARG	A	19	35.515	32.617	44.021	1.00	53.72	7
	ATOM	140	NH1	ARG	A	19	37.640	32.800	43.686	1.00	51.43	7
	ATOM	141	CZ	ARG	A	19	36.530	32.120	43.307	1.00	57.69	6
	ATOM	142	NE	ARG	A	19	36.207	31.186	42.351	1.00	42.98	7
25	ATOM	143	CD	ARG	A	19	37.338	31.011	41.450	1.00	46.84	6
	ATOM	144	CG	ARG	A	19	37.117	31.155	39.995	1.00	33.34	6
	ATOM	145	CB	ARG	A	19	35.800	30.421	39.724	1.00	26.86	6
	ATOM	146	CA	ARG	A	19	35.773	30.449	38.180	1.00	24.16	6
	ATOM	147	C	ARG	A	19	34.635	29.545	37.735	1.00	32.80	6
30	ATOM	148	O	ARG	A	19	34.691	28.447	38.295	1.00	38.37	8
	ATOM	149	N	GLY	A	20	33.659	29.890	36.943	1.00	26.10	7
	ATOM	150	CA	GLY	A	20	32.569	28.978	36.587	1.00	22.13	6
	ATOM	151	C	GLY	A	20	31.546	28.912	37.702	1.00	34.41	6
	ATOM	152	O	GLY	A	20	30.872	27.856	37.735	1.00	28.59	8
35	ATOM	153	N	ILE	A	21	31.493	29.934	38.591	1.00	29.96	7
	ATOM	154	CD1	ILE	A	21	33.459	29.632	41.814	1.00	41.54	6
	ATOM	155	CG1	ILE	A	21	32.100	29.052	41.506	1.00	25.19	6
	ATOM	156	CB	ILE	A	21	30.975	29.986	41.122	1.00	26.29	6
	ATOM	157	CG2	ILE	A	21	29.844	29.735	42.107	1.00	19.84	6
40	ATOM	158	CA	ILE	A	21	30.460	29.794	39.684	1.00	32.15	6
	ATOM	159	C	ILE	A	21	29.284	30.745	39.329	1.00	27.88	6
	ATOM	160	O	ILE	A	21	29.528	31.975	39.238	1.00	25.54	8
	ATOM	161	N	PHE	A	22	28.130	30.216	39.043	1.00	22.71	7
	ATOM	162	CD2	PHE	A	22	28.593	30.211	35.689	1.00	27.44	6
45	ATOM	163	CE2	PHE	A	22	29.621	30.567	34.823	1.00	24.36	6
	ATOM	164	CZ	PHE	A	22	29.741	31.905	34.446	1.00	33.93	6
	ATOM	165	CE1	PHE	A	22	28.872	32.884	34.911	1.00	27.82	6
	ATOM	166	CD1	PHE	A	22	27.870	32.510	35.793	1.00	28.92	6
	ATOM	167	CG	PHE	A	22	27.724	31.192	36.172	1.00	28.03	6
50	ATOM	168	CB	PHE	A	22	26.658	30.789	37.118	1.00	24.21	6
	ATOM	169	CA	PHE	A	22	26.950	30.969	38.613	1.00	26.09	6
	ATOM	170	C	PHE	A	22	25.683	30.711	39.409	1.00	25.39	6
	ATOM	171	O	PHE	A	22	24.665	31.302	38.981	1.00	24.97	8
	ATOM	172	N	GLY	A	23	25.607	29.924	40.467	1.00	18.81	7
55	ATOM	173	CA	GLY	A	23	24.363	29.724	41.148	1.00	18.46	6
	ATOM	174	C	GLY	A	23	23.503	28.543	40.757	1.00	19.87	6
	ATOM	175	O	GLY	A	23	22.414	28.258	41.288	1.00	21.97	8
	ATOM	176	N	ASN	A	24	24.176	27.813	39.877	1.00	24.80	7
	ATOM	177	ND2	ASN	A	24	24.193	25.603	36.454	1.00	54.67	7
60	ATOM	178	OD1	ASN	A	24	23.354	24.090	38.041	1.00	52.66	8
	ATOM	179	CG	ASN	A	24	24.034	25.056	37.655	1.00	54.67	6

338

	ATOM	180	CB	ASN	A	24	24.770	26.009	38.589	1.00	32.23	6
	ATOM	181	CA	ASN	A	24	23.593	26.534	39.395	1.00	25.92	6
	ATOM	182	C	ASN	A	24	23.179	25.638	40.552	1.00	25.32	6
	ATOM	183	O	ASN	A	24	23.976	25.322	41.465	1.00	30.34	8
5	ATOM	184	N	GLY	A	25	21.885	25.306	40.580	1.00	24.65	7
	ATOM	185	CA	GLY	A	25	21.465	24.504	41.725	1.00	28.29	6
	ATOM	186	C	GLY	A	25	20.845	25.160	42.938	1.00	26.14	6
	ATOM	187	O	GLY	A	25	20.160	24.516	43.717	1.00	27.35	8
	ATOM	188	N	ALA	A	26	21.025	26.469	43.065	1.00	33.36	7
10	ATOM	189	CB	ALA	A	26	21.389	28.357	44.440	1.00	22.66	6
	ATOM	190	CA	ALA	A	26	20.451	27.216	44.226	1.00	21.52	6
	ATOM	191	C	ALA	A	26	19.024	27.532	43.905	1.00	18.32	6
	ATOM	192	O	ALA	A	26	18.702	27.928	42.773	1.00	24.15	8
	ATOM	193	N	ARG	A	27	18.210	27.375	44.899	1.00	19.06	7
15	ATOM	194	NH2	ARG	A	27	15.995	22.073	47.281	1.00	46.56	7
	ATOM	195	NH1	ARG	A	27	16.803	22.004	45.047	1.00	39.77	7
	ATOM	196	CZ	ARG	A	27	16.017	22.485	46.012	1.00	48.33	6
	ATOM	197	NE	ARG	A	27	15.098	23.456	45.820	1.00	41.99	7
	ATOM	198	CD	ARG	A	27	15.075	24.160	44.559	1.00	36.91	6
20	ATOM	199	CG	ARG	A	27	16.301	25.064	44.358	1.00	29.21	6
	ATOM	200	CB	ARG	A	27	15.999	26.369	45.132	1.00	26.05	6
	ATOM	201	CA	ARG	A	27	16.785	27.590	44.764	1.00	19.90	6
	ATOM	202	C	ARG	A	27	16.462	28.820	45.623	1.00	24.82	6
	ATOM	203	O	ARG	A	27	16.484	28.798	46.855	1.00	23.36	8
25	ATOM	204	N	VAL	A	28	16.090	29.902	44.963	1.00	21.58	7
	ATOM	205	CG2	VAL	A	28	18.212	31.847	44.971	1.00	20.76	6
	ATOM	206	CG1	VAL	A	28	16.584	33.595	45.659	1.00	24.41	6
	ATOM	207	CB	VAL	A	28	16.756	32.246	44.948	1.00	18.33	6
	ATOM	208	CA	VAL	A	28	15.821	31.208	45.600	1.00	20.58	6
30	ATOM	209	C	VAL	A	28	14.369	31.568	45.504	1.00	16.41	6
	ATOM	210	O	VAL	A	28	13.904	31.628	44.344	1.00	22.07	8
	ATOM	211	N	ALA	A	29	13.724	31.792	46.617	1.00	15.89	7
	ATOM	212	CB	ALA	A	29	11.536	31.675	47.718	1.00	16.94	6
	ATOM	213	CA	ALA	A	29	12.322	32.248	46.580	1.00	21.50	6
35	ATOM	214	C	ALA	A	29	12.353	33.820	46.734	1.00	19.32	6
	ATOM	215	O	ALA	A	29	13.042	34.312	47.649	1.00	19.70	8
	ATOM	216	N	VAL	A	30	11.770	34.530	45.806	1.00	18.83	7
	ATOM	217	CG2	VAL	A	30	13.356	36.406	44.142	1.00	17.28	6
	ATOM	218	CG1	VAL	A	30	11.680	38.150	44.538	1.00	19.61	6
40	ATOM	219	CB	VAL	A	30	11.885	36.649	44.450	1.00	19.02	6
	ATOM	220	CA	VAL	A	30	11.590	35.993	45.824	1.00	21.94	6
	ATOM	221	C	VAL	A	30	10.211	36.329	46.406	1.00	17.79	6
	ATOM	222	O	VAL	A	30	9.239	36.104	45.639	1.00	16.80	8
	ATOM	223	N	LEU	A	31	10.136	36.740	47.677	1.00	16.21	7
45	ATOM	224	CD2	LEU	A	31	8.443	35.115	51.734	1.00	18.64	6
	ATOM	225	CD1	LEU	A	31	9.392	34.230	49.510	1.00	18.41	6
	ATOM	226	CG	LEU	A	31	8.513	35.233	50.228	1.00	27.95	6
	ATOM	227	CB	LEU	A	31	8.841	36.689	49.787	1.00	17.41	6
	ATOM	228	CA	LEU	A	31	8.837	37.091	48.332	1.00	17.17	6
50	ATOM	229	C	LEU	A	31	8.609	38.573	48.053	1.00	23.39	6
	ATOM	230	O	LEU	A	31	9.245	39.436	48.649	1.00	19.56	8
	ATOM	231	N	ASP	A	32	7.756	38.918	47.142	1.00	20.33	7
	ATOM	232	OD2	ASP	A	32	8.509	42.872	45.463	1.00	17.46	8
	ATOM	233	OD1	ASP	A	32	10.355	42.272	46.272	1.00	18.58	8
55	ATOM	234	CG	ASP	A	32	9.249	41.959	45.903	1.00	17.91	6
	ATOM	235	CB	ASP	A	32	8.780	40.509	45.770	1.00	17.55	6
	ATOM	236	CA	ASP	A	32	7.544	40.265	46.640	1.00	18.05	6
	ATOM	237	C	ASP	A	32	6.259	40.407	45.874	1.00	16.34	6
	ATOM	238	O	ASP	A	32	5.265	39.719	46.233	1.00	18.95	8
60	ATOM	239	N	THR	A	33	6.345	41.337	44.922	1.00	18.08	7
	ATOM	240	CG2	THR	A	33	5.111	44.100	44.539	1.00	15.20	6

339

	ATOM	241	OG1	THR	A	33	6.078	43.108	42.626	1.00	15.34	8
	ATOM	242	CB	THR	A	33	5.050	42.995	43.536	1.00	17.62	6
	ATOM	243	CA	THR	A	33	5.068	41.559	44.165	1.00	19.10	6
	ATOM	244	C	THR	A	33	4.876	40.503	43.046	1.00	21.43	6
5	ATOM	245	O	THR	A	33	3.956	40.703	42.210	1.00	19.77	8
	ATOM	246	N	GLY	A	34	5.747	39.519	42.979	1.00	19.23	7
	ATOM	247	CA	GLY	A	34	5.694	38.503	41.928	1.00	18.38	6
	ATOM	248	C	GLY	A	34	6.872	38.646	41.034	1.00	17.22	6
	ATOM	249	O	GLY	A	34	7.711	39.459	41.383	1.00	18.99	8
10	ATOM	250	N	ILE	A	35	6.974	37.882	39.956	1.00	17.46	7
	ATOM	251	CD1	ILE	A	35	10.899	35.757	40.596	1.00	15.13	6
	ATOM	252	CG1	ILE	A	35	9.791	36.828	40.462	1.00	14.72	6
	ATOM	253	CB	ILE	A	35	9.166	36.970	39.068	1.00	15.03	6
	ATOM	254	CG2	ILE	A	35	10.243	37.326	38.068	1.00	15.97	6
15	ATOM	255	CA	ILE	A	35	8.048	37.960	38.978	1.00	14.81	6
	ATOM	256	C	ILE	A	35	7.360	37.965	37.617	1.00	17.66	6
	ATOM	257	O	ILE	A	35	6.554	37.071	37.431	1.00	21.48	8
	ATOM	258	N	ALA	A	37	7.565	38.985	36.818	1.00	17.09	7
	ATOM	259	CB	ALA	A	37	6.974	40.415	34.895	1.00	19.79	6
20	ATOM	260	CA	ALA	A	37	6.929	39.026	35.522	1.00	19.65	6
	ATOM	261	C	ALA	A	37	7.799	38.217	34.551	1.00	17.88	6
	ATOM	262	O	ALA	A	37	9.037	38.066	34.604	1.00	21.23	8
	ATOM	263	N	SER	A	38	7.062	37.689	33.589	1.00	16.80	7
	ATOM	264	OG	SER	A	38	7.219	35.805	30.632	1.00	30.69	8
25	ATOM	265	CB	SER	A	38	6.656	36.129	31.852	1.00	24.32	6
	ATOM	266	CA	SER	A	38	7.794	36.946	32.527	1.00	20.02	6
	ATOM	267	C	SER	A	38	8.554	38.064	31.824	1.00	20.83	6
	ATOM	268	O	SER	A	38	8.026	39.138	31.556	1.00	21.16	8
	ATOM	269	N	HIS	A	39	9.788	37.876	31.449	1.00	16.67	7
30	ATOM	270	CD2	HIS	A	39	11.839	42.154	31.855	1.00	18.50	6
	ATOM	271	NE2	HIS	A	39	12.849	42.828	31.229	1.00	17.78	7
	ATOM	272	CE1	HIS	A	39	13.757	41.990	30.654	1.00	19.11	6
	ATOM	273	ND1	HIS	A	39	13.250	40.817	30.899	1.00	18.95	7
	ATOM	274	CG	HIS	A	39	12.108	40.809	31.630	1.00	18.98	6
35	ATOM	275	CB	HIS	A	39	11.359	39.557	32.049	1.00	18.97	6
	ATOM	276	CA	HIS	A	39	10.744	38.721	30.858	1.00	19.12	6
	ATOM	277	C	HIS	A	39	11.775	37.948	30.062	1.00	17.80	6
	ATOM	278	O	HIS	A	39	12.355	37.014	30.570	1.00	20.73	8
	ATOM	279	N	PRO	A	40	12.200	38.418	28.889	1.00	21.00	7
40	ATOM	280	CG	PRO	A	40	12.293	39.449	26.786	1.00	21.21	6
	ATOM	281	CD	PRO	A	40	11.597	39.542	28.113	1.00	18.96	6
	ATOM	282	CB	PRO	A	40	13.560	38.729	26.913	1.00	19.67	6
	ATOM	283	CA	PRO	A	40	13.254	37.823	28.100	1.00	22.54	6
	ATOM	284	C	PRO	A	40	14.534	37.614	28.909	1.00	24.98	6
45	ATOM	285	O	PRO	A	40	15.326	36.689	28.538	1.00	23.15	8
	ATOM	286	N	ASP	A	41	14.864	38.402	29.921	1.00	21.23	7
	ATOM	287	OD2	ASP	A	41	19.022	40.411	31.203	1.00	23.14	8
	ATOM	288	OD1	ASP	A	41	18.902	38.575	30.179	1.00	20.45	8
	ATOM	289	CG	ASP	A	41	18.278	39.474	30.706	1.00	21.49	6
50	ATOM	290	CB	ASP	A	41	16.801	39.675	30.849	1.00	17.52	6
	ATOM	291	CA	ASP	A	41	16.149	38.300	30.623	1.00	18.20	6
	ATOM	292	C	ASP	A	41	16.007	37.531	31.930	1.00	16.57	6
	ATOM	293	O	ASP	A	41	16.990	37.609	32.687	1.00	21.11	8
	ATOM	294	N	LEU	A	42	14.877	36.908	32.100	1.00	16.23	7
55	ATOM	295	CD2	LEU	A	42	15.154	37.970	35.800	1.00	20.71	6
	ATOM	296	CD1	LEU	A	42	12.728	38.634	35.680	1.00	18.04	6
	ATOM	297	CG	LEU	A	42	13.906	38.079	34.940	1.00	22.07	6
	ATOM	298	CB	LEU	A	42	13.573	36.743	34.250	1.00	19.04	6
	ATOM	299	CA	LEU	A	42	14.688	36.119	33.316	1.00	18.11	6
60	ATOM	300	C	LEU	A	42	14.147	34.706	33.035	1.00	22.16	6
	ATOM	301	O	LEU	A	42	13.321	34.478	32.117	1.00	24.54	8

340

	ATOM	302	N	ARG	A	43	14.426	33.731	33.856	1.00	20.59	7
	ATOM	303	NH2	ARG	A	43	16.861	27.990	36.107	1.00	53.82	7
	ATOM	304	NH1	ARG	A	43	14.504	27.483	36.114	1.00	58.81	7
	ATOM	305	CZ	ARG	A	43	15.623	27.968	35.534	1.00	59.96	6
5	ATOM	306	NE	ARG	A	43	15.539	28.580	34.285	1.00	59.26	7
	ATOM	307	CD	ARG	A	43	14.187	29.098	33.890	1.00	53.79	6
	ATOM	308	CG	ARG	A	43	14.538	30.144	32.891	1.00	38.80	6
	ATOM	309	CB	ARG	A	43	14.893	31.393	33.636	1.00	20.63	6
	ATOM	310	CA	ARG	A	43	13.780	32.413	33.764	1.00	21.97	6
10	ATOM	311	C	ARG	A	43	13.120	32.158	35.092	1.00	20.02	6
	ATOM	312	O	ARG	A	43	13.858	32.194	36.102	1.00	24.03	8
	ATOM	313	N	ILE	A	44	11.867	31.959	35.226	1.00	20.63	7
	ATOM	314	CD1	ILE	A	44	8.902	34.679	35.796	1.00	25.57	6
	ATOM	315	CG1	ILE	A	44	10.068	33.881	36.368	1.00	29.55	6
15	ATOM	316	CB	ILE	A	44	9.746	32.360	36.490	1.00	24.21	6
	ATOM	317	CG2	ILE	A	44	8.902	31.922	37.662	1.00	21.80	6
	ATOM	318	CA	ILE	A	44	11.103	31.670	36.445	1.00	20.36	6
	ATOM	319	C	ILE	A	44	10.838	30.166	36.550	1.00	28.98	6
	ATOM	320	O	ILE	A	44	10.177	29.571	35.695	1.00	23.55	8
20	ATOM	321	N	ALA	A	45	11.322	29.549	37.602	1.00	27.19	7
	ATOM	322	CB	ALA	A	45	12.254	27.427	38.711	1.00	18.19	6
	ATOM	323	CA	ALA	A	45	11.176	28.111	37.907	1.00	25.70	6
	ATOM	324	C	ALA	A	45	9.799	27.798	38.418	1.00	25.04	6
	ATOM	325	O	ALA	A	45	9.394	26.706	38.033	1.00	28.94	8
25	ATOM	326	N	GLY	A	46	9.044	28.597	39.089	1.00	20.03	7
	ATOM	327	CA	GLY	A	46	7.719	28.282	39.555	1.00	16.95	6
	ATOM	328	C	GLY	A	46	7.400	29.295	40.624	1.00	22.67	6
	ATOM	329	O	GLY	A	46	8.103	30.327	40.564	1.00	21.98	8
	ATOM	330	N	GLY	A	47	6.408	29.068	41.382	1.00	22.31	7
30	ATOM	331	CA	GLY	A	47	6.038	30.017	42.427	1.00	21.33	6
	ATOM	332	C	GLY	A	47	4.601	29.839	42.841	1.00	25.87	6
	ATOM	333	O	GLY	A	47	3.918	28.882	42.428	1.00	25.43	8
	ATOM	334	N	ALA	A	48	4.055	30.737	43.620	1.00	20.53	7
	ATOM	335	CB	ALA	A	48	2.815	29.944	45.442	1.00	20.90	6
35	ATOM	336	CA	ALA	A	48	2.713	30.745	44.144	1.00	20.50	6
	ATOM	337	C	ALA	A	48	2.326	32.203	44.460	1.00	29.20	6
	ATOM	338	O	ALA	A	48	3.178	33.083	44.532	1.00	25.97	8
	ATOM	339	N	SER	A	49	1.068	32.454	44.688	1.00	22.19	7
	ATOM	340	OG	SER	A	49	-0.986	35.495	44.409	1.00	27.17	8
40	ATOM	341	CB	SER	A	49	-0.441	34.225	43.938	1.00	26.70	6
	ATOM	342	CA	SER	A	49	0.478	33.712	45.013	1.00	22.03	6
	ATOM	343	C	SER	A	49	-0.307	33.577	46.315	1.00	31.92	6
	ATOM	344	O	SER	A	49	-1.067	32.591	46.360	1.00	26.97	8
	ATOM	345	N	PHE	A	50	-0.097	34.588	47.147	1.00	22.91	7
45	ATOM	346	CD2	PHE	A	50	-0.049	32.109	50.111	1.00	31.06	6
	ATOM	347	CE2	PHE	A	50	0.409	30.786	49.993	1.00	23.47	6
	ATOM	348	CZ	PHE	A	50	1.692	30.585	49.509	1.00	26.37	6
	ATOM	349	CE1	PHE	A	50	2.459	31.650	49.044	1.00	27.36	6
	ATOM	350	CD1	PHE	A	50	1.909	32.920	49.123	1.00	25.18	6
50	ATOM	351	CG	PHE	A	50	0.659	33.206	49.640	1.00	27.18	6
	ATOM	352	CB	PHE	A	50	0.068	34.581	49.654	1.00	20.39	6
	ATOM	353	CA	PHE	A	50	-0.814	34.627	48.416	1.00	20.79	6
	ATOM	354	C	PHE	A	50	-1.699	35.845	48.217	1.00	26.50	6
	ATOM	355	O	PHE	A	50	-2.095	36.380	49.255	1.00	33.21	8
55	ATOM	356	N	ILE	A	51	-2.067	36.337	47.068	1.00	25.81	7
	ATOM	357	CD1	ILE	A	51	-0.964	39.394	48.263	1.00	25.15	6
	ATOM	358	CG1	ILE	A	51	-0.838	39.160	46.744	1.00	26.10	6
	ATOM	359	CB	ILE	A	51	-2.155	38.659	46.174	1.00	28.46	6
	ATOM	360	CG2	ILE	A	51	-2.994	39.906	45.884	1.00	26.35	6
60	ATOM	361	CA	ILE	A	51	-2.870	37.563	46.980	1.00	25.17	6
	ATOM	362	C	ILE	A	51	-4.111	37.059	46.276	1.00	22.13	6

341

	ATOM	363	O	ILE	A	51	-4.019	36.809	45.075	1.00	26.47	8
	ATOM	364	N	SER	A	52	-5.211	36.990	46.985	1.00	31.96	7
	ATOM	365	OG	SER	A	52	-7.326	37.187	48.213	1.00	55.96	8
	ATOM	366	CB	SER	A	52	-7.637	36.283	47.168	1.00	40.98	6
5	ATOM	367	CA	SER	A	52	-6.416	36.494	46.288	1.00	36.15	6
	ATOM	368	C	SER	A	52	-6.840	37.320	45.088	1.00	41.46	6
	ATOM	369	O	SER	A	52	-7.334	36.657	44.131	1.00	42.48	8
	ATOM	370	N	SER	A	53	-6.711	38.640	45.097	1.00	34.99	7
	ATOM	371	OG	SER	A	53	-6.064	41.220	44.420	1.00	45.24	8
10	ATOM	372	CB	SER	A	53	-7.345	40.753	44.027	1.00	36.41	6
	ATOM	373	CA	SER	A	53	-7.166	39.272	43.832	1.00	32.42	6
	ATOM	374	C	SER	A	53	-6.198	39.008	42.704	1.00	28.79	6
	ATOM	375	O	SER	A	53	-6.518	39.427	41.610	1.00	30.59	8
	ATOM	376	N	GLU	A	54	-5.089	38.335	42.931	1.00	26.60	7
15	ATOM	377	OE2	GLU	A	54	-2.266	42.297	42.536	1.00	28.17	8
	ATOM	378	OE1	GLU	A	54	-0.866	41.124	41.290	1.00	25.34	8
	ATOM	379	CD	GLU	A	54	-1.988	41.335	41.716	1.00	26.67	6
	ATOM	380	CG	GLU	A	54	-3.245	40.511	41.554	1.00	33.12	6
	ATOM	381	CB	GLU	A	54	-2.993	39.046	41.906	1.00	30.53	6
20	ATOM	382	CA	GLU	A	54	-4.147	38.053	41.836	1.00	27.17	6
	ATOM	383	C	GLU	A	54	-3.550	36.669	41.985	1.00	29.10	6
	ATOM	384	O	GLU	A	54	-2.499	36.360	42.543	1.00	31.16	8
	ATOM	385	N	PRO	A	55	-4.303	35.698	41.531	1.00	28.22	7
	ATOM	386	CG	PRO	A	55	-6.256	34.510	40.919	1.00	32.87	6
25	ATOM	387	CD	PRO	A	55	-5.638	35.901	40.877	1.00	27.93	6
	ATOM	388	CB	PRO	A	55	-5.108	33.565	40.980	1.00	25.50	6
	ATOM	389	CA	PRO	A	55	-3.921	34.295	41.596	1.00	27.69	6
	ATOM	390	C	PRO	A	55	-2.652	33.893	40.869	1.00	26.18	6
	ATOM	391	O	PRO	A	55	-2.111	32.861	41.284	1.00	29.26	8
30	ATOM	392	N	SER	A	57	-2.177	34.589	39.865	1.00	23.03	7
	ATOM	393	OG	SER	A	57	0.204	34.676	37.165	1.00	24.28	8
	ATOM	394	CB	SER	A	57	-1.012	34.882	37.811	1.00	17.78	6
	ATOM	395	CA	SER	A	57	-0.933	34.228	39.178	1.00	17.61	6
	ATOM	396	C	SER	A	57	0.231	34.769	40.022	1.00	23.28	6
35	ATOM	397	O	SER	A	57	0.077	35.788	40.730	1.00	23.01	8
	ATOM	398	N	TYR	A	58	1.401	34.208	39.978	1.00	21.42	7
	ATOM	399	OH	TYR	A	58	5.286	30.151	36.865	1.00	33.08	8
	ATOM	400	CD2	TYR	A	58	4.751	33.134	38.858	1.00	20.82	6
	ATOM	401	CE2	TYR	A	58	5.242	32.389	37.792	1.00	27.67	6
40	ATOM	402	CZ	TYR	A	58	4.847	31.036	37.806	1.00	30.71	6
	ATOM	403	CE1	TYR	A	58	4.098	30.504	38.847	1.00	24.64	6
	ATOM	404	CD1	TYR	A	58	3.650	31.337	39.884	1.00	30.01	6
	ATOM	405	CG	TYR	A	58	3.956	32.697	39.898	1.00	24.45	6
	ATOM	406	CB	TYR	A	58	3.496	33.547	41.049	1.00	19.56	6
45	ATOM	407	CA	TYR	A	58	2.579	34.707	40.656	1.00	22.41	6
	ATOM	408	C	TYR	A	58	3.245	35.769	39.795	1.00	18.11	6
	ATOM	409	O	TYR	A	58	4.272	36.323	40.134	1.00	19.48	8
	ATOM	410	N	HIS	A	59	2.819	36.120	38.608	1.00	19.19	7
	ATOM	411	CD2	HIS	A	59	2.574	34.690	35.084	1.00	24.45	6
50	ATOM	412	NE2	HIS	A	59	3.570	33.918	34.542	1.00	23.56	7
	ATOM	413	CE1	HIS	A	59	4.820	34.391	34.635	1.00	23.74	6
	ATOM	414	ND1	HIS	A	59	4.689	35.505	35.318	1.00	27.94	7
	ATOM	415	CG	HIS	A	59	3.333	35.753	35.529	1.00	23.77	6
	ATOM	416	CB	HIS	A	59	2.907	37.006	36.276	1.00	23.35	6
55	ATOM	417	CA	HIS	A	59	3.464	37.096	37.717	1.00	23.68	6
	ATOM	418	C	HIS	A	59	3.223	38.478	38.330	1.00	16.77	6
	ATOM	419	O	HIS	A	59	2.112	38.802	38.813	1.00	20.69	8
	ATOM	420	N	ASP	A	60	4.262	39.225	38.217	1.00	17.78	7
	ATOM	421	OD2	ASP	A	60	7.207	42.684	39.352	1.00	16.87	8
60	ATOM	422	OD1	ASP	A	60	5.224	42.870	40.299	1.00	17.98	8
	ATOM	423	CG	ASP	A	60	6.005	42.319	39.583	1.00	15.82	6

342

	ATOM	424	CB	ASP	A	60	5.713	41.108	38.718	1.00	20.17	6
	ATOM	425	CA	ASP	A	60	4.257	40.615	38.746	1.00	19.60	6
	ATOM	426	C	ASP	A	60	3.449	41.628	37.887	1.00	16.78	6
	ATOM	427	O	ASP	A	60	3.755	41.641	36.688	1.00	17.17	8
5	ATOM	428	N	ASN	A	61	2.553	42.321	38.565	1.00	16.17	7
	ATOM	429	ND2	ASN	A	61	-0.712	41.216	38.409	1.00	21.25	7
	ATOM	430	OD1	ASN	A	61	0.074	41.753	36.354	1.00	22.89	8
	ATOM	431	CG	ASN	A	61	-0.126	42.022	37.543	1.00	19.95	6
	ATOM	432	CB	ASN	A	61	0.343	43.358	38.057	1.00	18.61	6
10	ATOM	433	CA	ASN	A	61	1.837	43.400	37.853	1.00	18.92	6
	ATOM	434	C	ASN	A	61	2.346	44.793	38.274	1.00	22.66	6
	ATOM	435	O	ASN	A	61	1.893	45.845	37.801	1.00	23.21	8
	ATOM	436	N	ASN	A	62	3.297	44.887	39.186	1.00	19.85	7
	ATOM	437	ND2	ASN	A	62	3.761	48.155	42.016	1.00	22.91	7
15	ATOM	438	OD1	ASN	A	62	5.928	47.387	41.972	1.00	21.51	8
	ATOM	439	CG	ASN	A	62	4.708	47.221	41.809	1.00	24.07	6
	ATOM	440	CB	ASN	A	62	4.074	45.934	41.266	1.00	15.90	6
	ATOM	441	CA	ASN	A	62	3.942	46.038	39.781	1.00	17.18	6
	ATOM	442	C	ASN	A	62	5.262	46.370	39.149	1.00	21.56	6
20	ATOM	443	O	ASN	A	62	5.450	47.489	38.652	1.00	23.34	8
	ATOM	444	N	GLY	A	63	6.219	45.499	39.274	1.00	16.07	7
	ATOM	445	CA	GLY	A	63	7.560	45.696	38.775	1.00	15.56	6
	ATOM	446	C	GLY	A	63	8.566	45.526	39.928	1.00	13.16	6
	ATOM	447	O	GLY	A	63	9.705	45.220	39.576	1.00	14.42	8
25	ATOM	448	N	HIS	A	64	8.181	45.732	41.170	1.00	14.55	7
	ATOM	449	CD2	HIS	A	64	9.944	47.365	45.114	1.00	19.41	6
	ATOM	450	NE2	HIS	A	64	10.615	47.068	46.239	1.00	17.69	7
	ATOM	451	CE1	HIS	A	64	10.371	45.792	46.555	1.00	17.59	6
	ATOM	452	ND1	HIS	A	64	9.605	45.312	45.607	1.00	19.22	7
30	ATOM	453	CG	HIS	A	64	9.334	46.232	44.659	1.00	17.77	6
	ATOM	454	CB	HIS	A	64	8.428	45.991	43.484	1.00	13.22	6
	ATOM	455	CA	HIS	A	64	9.195	45.658	42.241	1.00	17.90	6
	ATOM	456	C	HIS	A	64	9.902	44.259	42.331	1.00	17.60	6
	ATOM	457	O	HIS	A	64	11.161	44.161	42.393	1.00	15.99	8
35	ATOM	458	N	GLY	A	65	9.081	43.180	42.309	1.00	16.44	7
	ATOM	459	CA	GLY	A	65	9.616	41.816	42.380	1.00	14.82	6
	ATOM	460	C	GLY	A	65	10.479	41.481	41.172	1.00	14.51	6
	ATOM	461	O	GLY	A	65	11.471	40.769	41.349	1.00	17.10	8
	ATOM	462	N	THR	A	66	10.099	41.938	39.997	1.00	14.08	7
40	ATOM	463	CG2	THR	A	66	10.799	41.935	36.263	1.00	16.28	6
	ATOM	464	OG1	THR	A	66	8.783	41.636	37.548	1.00	16.38	8
	ATOM	465	CB	THR	A	66	10.092	42.160	37.567	1.00	13.88	6
	ATOM	466	CA	THR	A	66	10.851	41.608	38.787	1.00	11.82	6
	ATOM	467	C	THR	A	66	12.223	42.209	38.848	1.00	17.20	6
45	ATOM	468	O	THR	A	66	13.251	41.729	38.360	1.00	15.82	8
	ATOM	469	N	HIS	A	67	12.283	43.430	39.440	1.00	16.72	7
	ATOM	470	CD2	HIS	A	67	14.672	47.526	38.936	1.00	14.06	6
	ATOM	471	NE2	HIS	A	67	15.894	48.068	39.341	1.00	15.93	7
	ATOM	472	CE1	HIS	A	67	16.222	47.455	40.502	1.00	16.28	6
50	ATOM	473	ND1	HIS	A	67	15.270	46.657	40.870	1.00	14.20	7
	ATOM	474	CG	HIS	A	67	14.288	46.658	39.897	1.00	13.11	6
	ATOM	475	CB	HIS	A	67	13.142	45.733	40.058	1.00	13.83	6
	ATOM	476	CA	HIS	A	67	13.524	44.275	39.602	1.00	17.85	6
	ATOM	477	C	HIS	A	67	14.489	43.467	40.555	1.00	12.74	6
55	ATOM	478	O	HIS	A	67	15.676	43.217	40.217	1.00	14.79	8
	ATOM	479	N	VAL	A	68	13.875	43.184	41.692	1.00	15.52	7
	ATOM	480	CG2	VAL	A	68	13.554	43.532	44.544	1.00	16.01	6
	ATOM	481	CG1	VAL	A	68	14.397	41.111	44.868	1.00	15.56	6
	ATOM	482	CB	VAL	A	68	13.732	42.126	43.930	1.00	17.25	6
60	ATOM	483	CA	VAL	A	68	14.631	42.373	42.702	1.00	18.13	6
	ATOM	484	C	VAL	A	68	15.115	41.029	42.063	1.00	13.97	6

343

	ATOM	485	O	VAL	A	68	16.303	40.718	42.241	1.00	15.56	8
	ATOM	486	N	ALA	A	69	14.226	40.381	41.343	1.00	16.97	7
	ATOM	487	CB	ALA	A	69	13.385	38.483	40.044	1.00	15.14	6
	ATOM	488	CA	ALA	A	69	14.625	39.104	40.683	1.00	20.11	6
5	ATOM	489	C	ALA	A	69	15.800	39.240	39.746	1.00	19.97	6
	ATOM	490	O	ALA	A	69	16.716	38.370	39.765	1.00	18.07	8
	ATOM	491	N	GLY	A	70	15.860	40.297	38.929	1.00	16.08	7
	ATOM	492	CA	GLY	A	70	16.915	40.521	37.962	1.00	13.42	6
	ATOM	493	C	GLY	A	70	18.248	40.803	38.624	1.00	17.11	6
10	ATOM	494	O	GLY	A	70	19.301	40.458	38.069	1.00	18.05	8
	ATOM	495	N	THR	A	71	18.251	41.364	39.834	1.00	16.82	7
	ATOM	496	CG2	THR	A	71	20.803	42.713	42.461	1.00	11.71	6
	ATOM	497	OG1	THR	A	71	19.044	43.833	41.152	1.00	19.96	8
	ATOM	498	CB	THR	A	71	19.494	42.605	41.692	1.00	17.79	6
15	ATOM	499	CA	THR	A	71	19.570	41.620	40.463	1.00	18.16	6
	ATOM	500	C	THR	A	71	20.085	40.254	40.907	1.00	16.28	6
	ATOM	501	O	THR	A	71	21.302	40.022	40.823	1.00	20.35	8
	ATOM	502	N	ILE	A	72	19.224	39.377	41.381	1.00	17.87	7
	ATOM	503	CD1	ILE	A	72	16.919	37.403	44.477	1.00	15.03	6
20	ATOM	504	CG1	ILE	A	72	18.141	37.904	43.767	1.00	16.72	6
	ATOM	505	CB	ILE	A	72	18.628	37.243	42.500	1.00	22.03	6
	ATOM	506	CG2	ILE	A	72	19.096	35.809	42.923	1.00	18.50	6
	ATOM	507	CA	ILE	A	72	19.708	38.025	41.767	1.00	18.21	6
	ATOM	508	C	ILE	A	72	20.158	37.194	40.536	1.00	18.25	6
25	ATOM	509	O	ILE	A	72	21.223	36.584	40.501	1.00	17.34	8
	ATOM	510	N	ALA	A	73	19.308	37.143	39.514	1.00	18.67	7
	ATOM	511	CB	ALA	A	73	18.850	34.961	38.811	1.00	21.72	6
	ATOM	512	CA	ALA	A	73	19.600	36.258	38.384	1.00	20.55	6
	ATOM	513	C	ALA	A	73	19.220	36.650	36.993	1.00	21.64	6
30	ATOM	514	O	ALA	A	73	18.847	35.677	36.292	1.00	21.02	8
	ATOM	515	N	ALA	A	74	19.351	37.891	36.551	1.00	19.57	7
	ATOM	516	CB	ALA	A	74	19.407	39.748	34.855	1.00	16.43	6
	ATOM	517	CA	ALA	A	74	19.129	38.268	35.176	1.00	17.51	6
	ATOM	518	C	ALA	A	74	20.182	37.387	34.423	1.00	21.22	6
35	ATOM	519	O	ALA	A	74	21.379	37.294	34.773	1.00	18.12	8
	ATOM	520	N	LEU	A	75	19.625	36.759	33.380	1.00	19.89	7
	ATOM	521	CD2	LEU	A	75	18.684	33.287	32.938	1.00	20.44	6
	ATOM	522	CD1	LEU	A	75	17.370	34.159	30.853	1.00	22.84	6
	ATOM	523	CG	LEU	A	75	18.279	34.390	32.036	1.00	23.72	6
40	ATOM	524	CB	LEU	A	75	19.491	35.129	31.487	1.00	22.59	6
	ATOM	525	CA	LEU	A	75	20.421	35.799	32.558	1.00	21.45	6
	ATOM	526	C	LEU	A	75	21.644	36.353	31.885	1.00	22.38	6
	ATOM	527	O	LEU	A	75	21.691	37.506	31.413	1.00	21.99	8
	ATOM	528	N	ASN	A	76	22.678	35.519	31.836	1.00	23.39	7
45	ATOM	529	ND2	ASN	A	76	27.453	34.761	31.699	1.00	31.91	7
	ATOM	530	OD1	ASN	A	76	26.466	36.466	30.730	1.00	26.97	8
	ATOM	531	CG	ASN	A	76	26.339	35.407	31.355	1.00	33.84	6
	ATOM	532	CB	ASN	A	76	24.992	34.941	31.890	1.00	18.81	6
	ATOM	533	CA	ASN	A	76	23.966	35.823	31.226	1.00	22.81	6
50	ATOM	534	C	ASN	A	76	23.762	35.565	29.728	1.00	32.51	6
	ATOM	535	O	ASN	A	76	23.757	34.402	29.350	1.00	27.52	8
	ATOM	536	N	ASN	A	77	23.499	36.553	28.890	1.00	29.68	7
	ATOM	537	ND2	ASN	A	77	19.501	36.639	28.267	1.00	20.91	7
	ATOM	538	OD1	ASN	A	77	21.260	38.058	28.176	1.00	23.61	8
55	ATOM	539	CG	ASN	A	77	20.739	36.958	28.001	1.00	23.21	6
	ATOM	540	CB	ASN	A	77	21.698	36.006	27.290	1.00	24.11	6
	ATOM	541	CA	ASN	A	77	23.184	36.392	27.455	1.00	29.10	6
	ATOM	542	C	ASN	A	77	23.597	37.625	26.699	1.00	23.45	6
	ATOM	543	O	ASN	A	77	24.554	38.269	27.092	1.00	26.46	8
60	ATOM	544	N	SER	A	78	22.917	37.914	25.631	1.00	23.85	7
	ATOM	545	OG	SER	A	78	23.826	38.128	22.933	1.00	51.66	8

344

	ATOM	546	CB	SER	A	78	22.726	38.836	23.468	1.00	38.99	6
	ATOM	547	CA	SER	A	78	23.343	39.124	24.902	1.00	28.32	6
	ATOM	548	C	SER	A	78	22.590	40.392	25.196	1.00	26.17	6
	ATOM	549	O	SER	A	78	22.848	41.406	24.556	1.00	30.79	8
5	ATOM	550	N	ILE	A	79	21.553	40.260	25.994	1.00	26.87	7
	ATOM	551	CD1	ILE	A	79	17.234	39.484	26.505	1.00	22.48	6
	ATOM	552	CG1	ILE	A	79	18.723	39.666	26.593	1.00	23.59	6
	ATOM	553	CB	ILE	A	79	19.291	40.851	25.835	1.00	29.56	6
	ATOM	554	CG2	ILE	A	79	19.401	40.371	24.400	1.00	25.83	6
10	ATOM	555	CA	ILE	A	79	20.675	41.390	26.218	1.00	22.47	6
	ATOM	556	C	ILE	A	79	20.590	41.758	27.679	1.00	22.23	6
	ATOM	557	O	ILE	A	79	21.096	41.041	28.498	1.00	21.05	8
	ATOM	558	N	GLY	A	80	19.921	42.847	27.901	1.00	21.78	7
	ATOM	559	CA	GLY	A	80	19.579	43.296	29.237	1.00	20.74	6
15	ATOM	560	C	GLY	A	80	20.731	43.409	30.215	1.00	22.30	6
	ATOM	561	O	GLY	A	80	21.767	43.988	29.848	1.00	24.00	8
	ATOM	562	N	VAL	A	81	20.534	42.884	31.415	1.00	20.72	7
	ATOM	563	CG2	VAL	A	81	19.687	43.194	34.148	1.00	16.10	6
	ATOM	564	CG1	VAL	A	81	20.666	45.283	33.070	1.00	19.66	6
20	ATOM	565	CB	VAL	A	81	20.938	43.844	33.561	1.00	21.57	6
	ATOM	566	CA	VAL	A	81	21.616	43.067	32.414	1.00	18.79	6
	ATOM	567	C	VAL	A	81	22.121	41.681	32.721	1.00	24.48	6
	ATOM	568	O	VAL	A	81	21.953	40.670	32.065	1.00	22.82	8
	ATOM	569	N	LEU	A	82	22.797	41.495	33.827	1.00	26.20	7
25	ATOM	570	CD2	LEU	A	82	27.235	39.378	34.412	1.00	20.59	6
	ATOM	571	CD1	LEU	A	82	25.342	37.924	33.896	1.00	22.30	6
	ATOM	572	CG	LEU	A	82	25.740	39.235	34.558	1.00	22.25	6
	ATOM	573	CB	LEU	A	82	24.947	40.464	34.054	1.00	20.75	6
	ATOM	574	CA	LEU	A	82	23.431	40.297	34.339	1.00	21.39	6
30	ATOM	575	C	LEU	A	82	23.171	40.165	35.847	1.00	19.49	6
	ATOM	576	O	LEU	A	82	23.528	41.144	36.502	1.00	23.34	8
	ATOM	577	N	GLY	A	83	22.671	39.066	36.348	1.00	20.69	7
	ATOM	578	CA	GLY	A	83	22.457	38.949	37.770	1.00	17.03	6
	ATOM	579	C	GLY	A	83	23.782	38.468	38.350	1.00	17.15	6
35	ATOM	580	O	GLY	A	83	24.759	38.085	37.729	1.00	17.70	8
	ATOM	581	N	VAL	A	84	23.723	38.456	39.683	1.00	21.38	7
	ATOM	582	CG2	VAL	A	84	24.533	39.699	42.307	1.00	17.59	6
	ATOM	583	CG1	VAL	A	84	25.675	37.585	42.933	1.00	18.61	6
	ATOM	584	CB	VAL	A	84	24.568	38.197	42.032	1.00	19.33	6
40	ATOM	585	CA	VAL	A	84	24.791	37.919	40.537	1.00	18.94	6
	ATOM	586	C	VAL	A	84	24.883	36.373	40.292	1.00	19.93	6
	ATOM	587	O	VAL	A	84	26.024	35.890	40.194	1.00	18.82	8
	ATOM	588	N	ALA	A	85	23.766	35.668	40.255	1.00	19.98	7
	ATOM	589	CB	ALA	A	85	23.136	33.645	41.452	1.00	16.16	6
45	ATOM	590	CA	ALA	A	85	23.717	34.185	40.149	1.00	23.42	6
	ATOM	591	C	ALA	A	85	22.819	33.819	38.945	1.00	15.76	6
	ATOM	592	O	ALA	A	85	21.669	33.420	39.123	1.00	17.91	8
	ATOM	593	N	PRO	A	86	23.320	34.080	37.739	1.00	19.61	7
	ATOM	594	CG	PRO	A	86	24.802	34.328	35.990	1.00	22.42	6
50	ATOM	595	CD	PRO	A	86	24.691	34.594	37.481	1.00	17.62	6
	ATOM	596	CB	PRO	A	86	23.412	34.286	35.395	1.00	18.97	6
	ATOM	597	CA	PRO	A	86	22.527	33.884	36.525	1.00	22.90	6
	ATOM	598	C	PRO	A	86	21.982	32.494	36.282	1.00	25.04	6
	ATOM	599	O	PRO	A	86	21.044	32.392	35.510	1.00	25.03	8
55	ATOM	600	N	SER	A	87	22.550	31.531	36.954	1.00	21.61	7
	ATOM	601	OG	SER	A	87	23.828	29.588	35.364	1.00	24.93	8
	ATOM	602	CB	SER	A	87	23.195	29.132	36.539	1.00	21.86	6
	ATOM	603	CA	SER	A	87	22.079	30.144	36.789	1.00	25.33	6
	ATOM	604	C	SER	A	87	21.253	29.730	37.973	1.00	27.17	6
60	ATOM	605	O	SER	A	87	20.806	28.602	37.975	1.00	26.19	8
	ATOM	606	N	ALA	A	88	20.892	30.516	38.966	1.00	23.05	7

345

	ATOM	607	CB	ALA	A	88	20.108	31.154	41.189	1.00	18.32	6
	ATOM	608	CA	ALA	A	88	20.051	30.084	40.053	1.00	22.79	6
	ATOM	609	C	ALA	A	88	18.628	29.760	39.608	1.00	21.41	6
	ATOM	610	O	ALA	A	88	18.106	30.259	38.608	1.00	25.76	8
5	ATOM	611	N	ASP	A	89	17.896	28.967	40.323	1.00	19.89	7
	ATOM	612	OD2	ASP	A	89	16.801	26.516	38.434	1.00	31.22	8
	ATOM	613	OD1	ASP	A	89	17.282	25.428	40.116	1.00	44.17	8
	ATOM	614	CG	ASP	A	89	16.662	26.363	39.689	1.00	32.29	6
	ATOM	615	CB	ASP	A	89	16.007	27.380	40.585	1.00	26.99	6
10	ATOM	616	CA	ASP	A	89	16.475	28.764	40.089	1.00	22.99	6
	ATOM	617	C	ASP	A	89	15.649	29.788	40.846	1.00	26.13	6
	ATOM	618	O	ASP	A	89	15.605	29.765	42.092	1.00	23.54	8
	ATOM	619	N	LEU	A	90	14.876	30.620	40.201	1.00	23.36	7
	ATOM	620	CD2	LEU	A	90	14.764	35.038	38.890	1.00	23.98	6
15	ATOM	621	CD1	LEU	A	90	15.677	34.244	41.144	1.00	23.31	6
	ATOM	622	CG	LEU	A	90	14.540	34.313	40.203	1.00	32.59	6
	ATOM	623	CB	LEU	A	90	14.110	32.873	39.878	1.00	29.22	6
	ATOM	624	CA	LEU	A	90	14.041	31.659	40.828	1.00	22.24	6
	ATOM	625	C	LEU	A	90	12.643	31.203	41.002	1.00	19.26	6
20	ATOM	626	O	LEU	A	90	12.017	30.724	40.038	1.00	20.76	8
	ATOM	627	N	TYR	A	91	12.125	31.476	42.174	1.00	17.22	7
	ATOM	628	OH	TYR	A	91	12.321	25.105	41.504	1.00	31.21	8
	ATOM	629	CD2	TYR	A	91	10.097	27.804	42.484	1.00	24.84	6
	ATOM	630	CE2	TYR	A	91	10.565	26.613	41.969	1.00	22.93	6
25	ATOM	631	CZ	TYR	A	91	11.917	26.318	42.020	1.00	31.94	6
	ATOM	632	CE1	TYR	A	91	12.863	27.261	42.476	1.00	23.17	6
	ATOM	633	CD1	TYR	A	91	12.382	28.442	43.022	1.00	19.76	6
	ATOM	634	CG	TYR	A	91	11.026	28.729	43.006	1.00	22.41	6
	ATOM	635	CB	TYR	A	91	10.551	30.077	43.551	1.00	22.69	6
30	ATOM	636	CA	TYR	A	91	10.755	31.167	42.437	1.00	17.72	6
	ATOM	637	C	TYR	A	91	10.023	32.465	42.832	1.00	21.10	6
	ATOM	638	O	TYR	A	91	10.483	33.128	43.740	1.00	21.02	8
	ATOM	639	N	ALA	A	92	8.955	32.776	42.133	1.00	23.09	7
	ATOM	640	CB	ALA	A	92	7.352	34.205	40.926	1.00	14.26	6
35	ATOM	641	CA	ALA	A	92	8.067	33.911	42.258	1.00	21.27	6
	ATOM	642	C	ALA	A	92	7.090	33.619	43.378	1.00	19.16	6
	ATOM	643	O	ALA	A	92	6.104	32.928	43.143	1.00	21.07	8
	ATOM	644	N	VAL	A	93	7.184	34.197	44.567	1.00	19.51	7
	ATOM	645	CG2	VAL	A	93	7.656	32.310	46.567	1.00	21.27	6
40	ATOM	646	CG1	VAL	A	93	5.678	33.194	47.960	1.00	19.09	6
	ATOM	647	CB	VAL	A	93	6.745	33.478	46.928	1.00	18.62	6
	ATOM	648	CA	VAL	A	93	6.141	34.036	45.629	1.00	17.35	6
	ATOM	649	C	VAL	A	93	5.534	35.446	45.836	1.00	18.48	6
	ATOM	650	O	VAL	A	93	6.166	36.320	46.491	1.00	17.69	8
45	ATOM	651	N	LYS	A	94	4.359	35.587	45.326	1.00	15.95	7
	ATOM	652	NZ	LYS	A	94	0.341	38.732	40.786	1.00	16.98	7
	ATOM	653	CE	LYS	A	94	1.380	38.435	41.794	1.00	17.51	6
	ATOM	654	CD	LYS	A	94	0.902	38.548	43.246	1.00	18.13	6
	ATOM	655	CG	LYS	A	94	1.857	38.317	44.368	1.00	19.09	6
50	ATOM	656	CB	LYS	A	94	2.668	37.038	44.233	1.00	16.57	6
	ATOM	657	CA	LYS	A	94	3.611	36.817	45.392	1.00	21.78	6
	ATOM	658	C	LYS	A	94	3.007	36.982	46.792	1.00	25.09	6
	ATOM	659	O	LYS	A	94	1.985	36.358	47.139	1.00	21.82	8
	ATOM	660	N	VAL	A	95	3.600	37.907	47.568	1.00	20.23	7
55	ATOM	661	CG2	VAL	A	95	5.283	38.661	50.019	1.00	20.17	6
	ATOM	662	CG1	VAL	A	95	4.360	36.294	49.917	1.00	25.66	6
	ATOM	663	CB	VAL	A	95	4.009	37.779	49.976	1.00	30.09	6
	ATOM	664	CA	VAL	A	95	3.030	38.216	48.885	1.00	21.11	6
	ATOM	665	C	VAL	A	95	2.623	39.696	48.987	1.00	24.66	6
60	ATOM	666	O	VAL	A	95	2.177	40.080	50.064	1.00	23.19	8
	ATOM	667	N	LEU	A	96	2.818	40.511	47.962	1.00	23.27	7

346

	ATOM	668	CD2	LEU	A	96	3.997	43.237	50.138	1.00	25.60	6
	ATOM	669	CD1	LEU	A	96	5.970	43.494	48.659	1.00	20.15	6
	ATOM	670	CG	LEU	A	96	4.751	42.698	48.975	1.00	22.84	6
	ATOM	671	CB	LEU	A	96	3.706	42.779	47.891	1.00	20.75	6
5	ATOM	672	CA	LEU	A	96	2.451	41.918	47.920	1.00	23.08	6
	ATOM	673	C	LEU	A	96	1.703	42.036	46.589	1.00	23.01	6
	ATOM	674	O	LEU	A	96	2.061	41.403	45.579	1.00	21.24	8
	ATOM	675	N	ASP	A	97	0.689	42.897	46.551	1.00	23.27	7
	ATOM	676	OD2	ASP	A	97	-2.600	45.183	46.914	1.00	34.41	8
10	ATOM	677	OD1	ASP	A	97	-0.584	45.765	46.103	1.00	29.86	8
	ATOM	678	CG	ASP	A	97	-1.488	44.950	46.240	1.00	30.57	6
	ATOM	679	CB	ASP	A	97	-1.555	43.475	45.731	1.00	26.33	6
	ATOM	680	CA	ASP	A	97	-0.137	43.056	45.358	1.00	23.04	6
	ATOM	681	C	ASP	A	97	0.478	44.050	44.362	1.00	19.75	6
15	ATOM	682	O	ASP	A	97	1.581	44.509	44.552	1.00	20.60	8
	ATOM	683	N	ARG	A	98	-0.293	44.333	43.361	1.00	21.05	7
	ATOM	684	NH2	ARG	A	98	-6.414	46.513	41.337	1.00	61.54	7
	ATOM	685	NH1	ARG	A	98	-5.383	46.580	39.258	1.00	61.06	7
	ATOM	686	CZ	ARG	A	98	-5.345	46.297	40.563	1.00	59.40	6
20	ATOM	687	NE	ARG	A	98	-4.287	45.797	41.191	1.00	43.41	7
	ATOM	688	CD	ARG	A	98	-3.085	45.642	40.374	1.00	30.97	6
	ATOM	689	CG	ARG	A	98	-2.099	45.874	41.477	1.00	23.76	6
	ATOM	690	CB	ARG	A	98	-0.838	45.175	41.048	1.00	25.56	6
	ATOM	691	CA	ARG	A	98	0.109	45.190	42.254	1.00	25.82	6
25	ATOM	692	C	ARG	A	98	0.420	46.628	42.667	1.00	23.93	6
	ATOM	693	O	ARG	A	98	1.088	47.281	41.838	1.00	23.91	8
	ATOM	694	N	ASN	A	99	-0.032	46.924	43.851	1.00	23.90	7
	ATOM	695	ND2	ASN	A	99	-1.713	49.748	42.838	1.00	28.85	7
	ATOM	696	OD1	ASN	A	99	-3.264	48.712	44.128	1.00	39.99	8
30	ATOM	697	CG	ASN	A	99	-2.098	49.125	43.955	1.00	32.07	6
	ATOM	698	CB	ASN	A	99	-1.056	48.862	45.047	1.00	28.96	6
	ATOM	699	CA	ASN	A	99	0.209	48.265	44.383	1.00	30.38	6
	ATOM	700	C	ASN	A	99	1.392	48.195	45.301	1.00	30.88	6
	ATOM	701	O	ASN	A	99	1.809	49.252	45.800	1.00	30.12	8
35	ATOM	702	N	GLY	A	100	1.910	47.022	45.541	1.00	24.81	7
	ATOM	703	CA	GLY	A	100	3.112	46.938	46.388	1.00	21.34	6
	ATOM	704	C	GLY	A	100	2.730	46.700	47.825	1.00	26.62	6
	ATOM	705	O	GLY	A	100	3.572	46.651	48.719	1.00	30.05	8
	ATOM	706	N	SER	A	101	1.455	46.465	47.998	1.00	25.04	7
40	ATOM	707	OG	SER	A	101	-1.086	47.063	50.195	1.00	52.71	8
	ATOM	708	CB	SER	A	101	-0.288	47.078	49.079	1.00	33.36	6
	ATOM	709	CA	SER	A	101	1.004	46.287	49.369	1.00	28.75	6
	ATOM	710	C	SER	A	101	0.669	44.899	49.843	1.00	37.54	6
	ATOM	711	O	SER	A	101	0.182	44.154	49.006	1.00	29.65	8
45	ATOM	712	N	GLY	A	102	0.852	44.455	51.064	1.00	35.37	7
	ATOM	713	CA	GLY	A	102	0.402	43.090	51.473	1.00	42.38	6
	ATOM	714	C	GLY	A	102	0.311	43.081	53.009	1.00	41.95	6
	ATOM	715	O	GLY	A	102	0.662	44.081	53.674	1.00	51.09	8
	ATOM	716	N	SER	A	103	-0.061	42.076	53.725	1.00	30.23	7
50	ATOM	717	OG	SER	A	103	-1.367	40.088	54.944	1.00	40.84	8
	ATOM	718	CB	SER	A	103	-1.220	41.179	55.778	1.00	31.04	6
	ATOM	719	CA	SER	A	103	-0.076	41.926	55.156	1.00	29.72	6
	ATOM	720	C	SER	A	103	1.057	41.013	55.610	1.00	31.65	6
	ATOM	721	O	SER	A	103	1.642	40.294	54.835	1.00	34.54	8
55	ATOM	722	N	LEU	A	104	1.319	41.101	56.870	1.00	28.22	7
	ATOM	723	CD2	LEU	A	104	4.090	42.177	60.461	1.00	51.24	6
	ATOM	724	CD1	LEU	A	104	4.621	42.281	58.095	1.00	41.73	6
	ATOM	725	CG	LEU	A	104	4.001	41.439	59.150	1.00	39.07	6
	ATOM	726	CB	LEU	A	104	2.654	40.887	58.817	1.00	38.11	6
60	ATOM	727	CA	LEU	A	104	2.397	40.307	57.444	1.00	31.06	6
	ATOM	728	C	LEU	A	104	1.894	38.866	57.408	1.00	35.45	6

347

	ATOM	729	O	LEU	A	104	2.809	38.009	57.345	1.00	34.06	8
	ATOM	730	N	ALA	A	105	0.578	38.666	57.355	1.00	30.73	7
	ATOM	731	CB	ALA	A	105	-1.345	37.170	57.302	1.00	28.85	6
	ATOM	732	CA	ALA	A	105	0.171	37.260	57.244	1.00	32.26	6
5	ATOM	733	C	ALA	A	105	0.492	36.695	55.838	1.00	30.41	6
	ATOM	734	O	ALA	A	105	0.790	35.495	55.764	1.00	26.17	8
	ATOM	735	N	SER	A	106	0.370	37.484	54.767	1.00	26.36	7
	ATOM	736	OG	SER	A	106	0.908	38.945	52.353	1.00	47.12	8
	ATOM	737	CB	SER	A	106	0.078	37.776	52.335	1.00	28.12	6
10	ATOM	738	CA	SER	A	106	0.695	36.929	53.429	1.00	27.72	6
	ATOM	739	C	SER	A	106	2.174	36.648	53.385	1.00	24.51	6
	ATOM	740	O	SER	A	106	2.586	35.664	52.760	1.00	25.93	8
	ATOM	741	N	VAL	A	107	3.021	37.452	54.025	1.00	22.96	7
	ATOM	742	CG2	VAL	A	107	5.113	39.633	53.921	1.00	23.30	6
15	ATOM	743	CG1	VAL	A	107	6.747	37.936	54.918	1.00	22.54	6
	ATOM	744	CB	VAL	A	107	5.292	38.352	54.742	1.00	23.47	6
	ATOM	745	CA	VAL	A	107	4.467	37.209	54.117	1.00	22.96	6
	ATOM	746	C	VAL	A	107	4.792	35.863	54.775	1.00	27.46	6
	ATOM	747	O	VAL	A	107	5.638	35.148	54.247	1.00	22.03	8
20	ATOM	748	N	ALA	A	108	4.152	35.572	55.895	1.00	26.22	7
	ATOM	749	CB	ALA	A	108	3.431	34.340	57.872	1.00	22.56	6
	ATOM	750	CA	ALA	A	108	4.291	34.320	56.623	1.00	22.04	6
	ATOM	751	C	ALA	A	108	3.862	33.098	55.769	1.00	23.82	6
	ATOM	752	O	ALA	A	108	4.541	32.073	55.760	1.00	25.45	8
25	ATOM	753	N	GLN	A	109	2.798	33.159	55.019	1.00	26.10	7
	ATOM	754	NE2	GLN	A	109	-1.990	31.648	53.180	1.00	56.51	7
	ATOM	755	OE1	GLN	A	109	-1.807	33.819	52.964	1.00	52.89	8
	ATOM	756	CD	GLN	A	109	-1.363	32.789	53.524	1.00	52.62	6
	ATOM	757	CG	GLN	A	109	-0.163	32.492	54.418	1.00	23.57	6
30	ATOM	758	CB	GLN	A	109	1.020	32.469	53.458	1.00	19.24	6
	ATOM	759	CA	GLN	A	109	2.302	32.153	54.141	1.00	21.53	6
	ATOM	760	C	GLN	A	109	3.302	31.924	53.060	1.00	23.82	6
	ATOM	761	O	GLN	A	109	3.633	30.801	52.709	1.00	24.29	8
	ATOM	762	N	GLY	A	110	3.955	32.956	52.566	1.00	26.56	7
35	ATOM	763	CA	GLY	A	110	5.010	32.793	51.539	1.00	21.54	6
	ATOM	764	C	GLY	A	110	6.193	32.057	52.065	1.00	18.77	6
	ATOM	765	O	GLY	A	110	6.890	31.359	51.328	1.00	20.70	8
	ATOM	766	N	ILE	A	111	6.506	32.348	53.333	1.00	19.34	7
	ATOM	767	CD1	ILE	A	111	8.879	34.550	56.483	1.00	19.97	6
40	ATOM	768	CG1	ILE	A	111	8.799	33.646	55.221	1.00	25.91	6
	ATOM	769	CB	ILE	A	111	8.041	32.300	55.338	1.00	21.06	6
	ATOM	770	CG2	ILE	A	111	9.069	31.422	56.004	1.00	19.42	6
	ATOM	771	CA	ILE	A	111	7.639	31.695	54.014	1.00	20.08	6
	ATOM	772	C	ILE	A	111	7.287	30.164	54.171	1.00	28.01	6
45	ATOM	773	O	ILE	A	111	8.174	29.356	53.925	1.00	19.72	8
	ATOM	774	N	GLU	A	112	6.057	29.853	54.534	1.00	26.23	7
	ATOM	775	OE2	GLU	A	112	5.242	26.589	57.599	1.00	55.41	8
	ATOM	776	OE1	GLU	A	112	5.307	28.380	59.130	1.00	58.68	8
	ATOM	777	CD	GLU	A	112	5.032	27.876	57.981	1.00	57.74	6
50	ATOM	778	CG	GLU	A	112	4.340	28.653	56.863	1.00	54.59	6
	ATOM	779	CB	GLU	A	112	4.264	28.406	55.355	1.00	26.07	6
	ATOM	780	CA	GLU	A	112	5.632	28.463	54.721	1.00	26.47	6
	ATOM	781	C	GLU	A	112	5.651	27.787	53.384	1.00	24.57	6
	ATOM	782	O	GLU	A	112	6.181	26.678	53.335	1.00	27.03	8
55	ATOM	783	N	TRP	A	113	5.345	28.415	52.295	1.00	20.47	7
	ATOM	784	CD2	TRP	A	113	5.939	28.229	47.577	1.00	23.15	6
	ATOM	785	CE3	TRP	A	113	7.244	28.726	47.644	1.00	22.83	6
	ATOM	786	CZ3	TRP	A	113	8.109	28.544	46.587	1.00	22.30	6
	ATOM	787	CH2	TRP	A	113	7.680	27.910	45.424	1.00	22.04	6
60	ATOM	788	CZ2	TRP	A	113	6.378	27.441	45.332	1.00	20.63	6
	ATOM	789	CE2	TRP	A	113	5.543	27.598	46.399	1.00	19.44	6

348

	ATOM	790	NE1	TRP	A	113	4.261	27.215	46.619	1.00	22.83	7
	ATOM	791	CD1	TRP	A	113	3.821	27.559	47.869	1.00	19.44	6
	ATOM	792	CG	TRP	A	113	4.847	28.192	48.511	1.00	20.85	6
	ATOM	793	CB	TRP	A	113	4.744	28.731	49.896	1.00	20.61	6
5	ATOM	794	CA	TRP	A	113	5.385	27.849	50.973	1.00	18.92	6
	ATOM	795	C	TRP	A	113	6.817	27.518	50.681	1.00	22.62	6
	ATOM	796	O	TRP	A	113	7.102	26.484	50.055	1.00	23.67	8
	ATOM	797	N	ALA	A	114	7.790	28.387	50.988	1.00	23.59	7
	ATOM	798	CB	ALA	A	114	10.199	29.314	50.947	1.00	21.64	6
10	ATOM	799	CA	ALA	A	114	9.208	28.145	50.684	1.00	20.45	6
	ATOM	800	C	ALA	A	114	9.720	26.925	51.508	1.00	24.40	6
	ATOM	801	O	ALA	A	114	10.656	26.271	51.084	1.00	22.37	8
	ATOM	802	N	ILE	A	115	9.263	26.665	52.696	1.00	21.85	7
	ATOM	803	CD1	ILE	A	115	8.887	27.137	57.080	1.00	21.98	6
15	ATOM	804	CG1	ILE	A	115	9.735	26.862	55.832	1.00	22.19	6
	ATOM	805	CB	ILE	A	115	9.187	25.725	54.945	1.00	36.67	6
	ATOM	806	CG2	ILE	A	115	9.445	24.332	55.597	1.00	26.39	6
	ATOM	807	CA	ILE	A	115	9.712	25.557	53.533	1.00	23.50	6
	ATOM	808	C	ILE	A	115	9.183	24.244	52.881	1.00	22.01	6
20	ATOM	809	O	ILE	A	115	9.979	23.385	52.509	1.00	23.68	8
	ATOM	810	N	ASN	A	116	7.904	24.294	52.591	1.00	23.13	7
	ATOM	811	ND2	ASN	A	116	5.718	22.906	53.985	1.00	35.86	7
	ATOM	812	OD1	ASN	A	116	4.028	23.976	53.170	1.00	43.01	8
	ATOM	813	CG	ASN	A	116	5.117	23.420	52.940	1.00	31.13	6
25	ATOM	814	CB	ASN	A	116	5.859	23.287	51.643	1.00	20.42	6
	ATOM	815	CA	ASN	A	116	7.327	23.166	51.910	1.00	19.55	6
	ATOM	816	C	ASN	A	116	7.917	22.893	50.561	1.00	29.30	6
	ATOM	817	O	ASN	A	116	7.758	21.709	50.183	1.00	30.79	8
	ATOM	818	N	ASN	A	117	8.452	23.795	49.801	1.00	22.00	7
30	ATOM	819	ND2	ASN	A	117	6.020	24.758	48.002	1.00	20.38	7
	ATOM	820	OD1	ASN	A	117	6.621	23.594	46.231	1.00	25.41	8
	ATOM	821	CG	ASN	A	117	6.944	24.266	47.222	1.00	21.80	6
	ATOM	822	CB	ASN	A	117	8.400	24.593	47.462	1.00	19.43	6
	ATOM	823	CA	ASN	A	117	8.993	23.648	48.467	1.00	18.42	6
35	ATOM	824	C	ASN	A	117	10.488	23.572	48.529	1.00	16.67	6
	ATOM	825	O	ASN	A	117	11.080	23.586	47.448	1.00	23.59	8
	ATOM	826	N	ASN	A	118	10.994	23.449	49.770	1.00	24.36	7
	ATOM	827	ND2	ASN	A	118	14.257	20.977	49.784	1.00	46.79	7
	ATOM	828	OD1	ASN	A	118	11.956	20.616	50.768	1.00	42.51	8
40	ATOM	829	CG	ASN	A	118	12.926	20.992	50.037	1.00	53.99	6
	ATOM	830	CB	ASN	A	118	12.676	22.017	48.931	1.00	40.09	6
	ATOM	831	CA	ASN	A	118	12.463	23.293	49.763	1.00	25.20	6
	ATOM	832	C	ASN	A	118	13.436	24.264	49.061	1.00	29.14	6
	ATOM	833	O	ASN	A	118	14.413	23.816	48.416	1.00	23.06	8
45	ATOM	834	N	MET	A	119	13.069	25.539	49.345	1.00	24.91	7
	ATOM	835	CE	MET	A	119	11.345	26.688	45.875	1.00	25.32	6
	ATOM	836	SD	MET	A	119	12.390	28.044	46.482	1.00	24.14	16
	ATOM	837	CG	MET	A	119	11.874	27.979	48.232	1.00	19.15	6
	ATOM	838	CB	MET	A	119	13.167	27.925	49.032	1.00	21.49	6
50	ATOM	839	CA	MET	A	119	13.931	26.603	48.812	1.00	20.74	6
	ATOM	840	C	MET	A	119	15.198	26.587	49.594	1.00	19.07	6
	ATOM	841	O	MET	A	119	15.184	26.188	50.752	1.00	23.02	8
	ATOM	842	N	HIS	A	120	16.296	27.065	49.124	1.00	18.99	7
	ATOM	843	CD2	HIS	A	120	18.647	24.610	49.083	1.00	30.88	6
55	ATOM	844	NE2	HIS	A	120	18.706	23.671	48.118	1.00	24.71	7
	ATOM	845	CE1	HIS	A	120	18.992	24.314	46.957	1.00	28.15	6
	ATOM	846	ND1	HIS	A	120	19.000	25.611	47.103	1.00	29.83	7
	ATOM	847	CG	HIS	A	120	18.816	25.840	48.415	1.00	26.54	6
	ATOM	848	CB	HIS	A	120	18.805	27.181	49.043	1.00	20.56	6
60	ATOM	849	CA	HIS	A	120	17.517	27.249	49.902	1.00	19.50	6
	ATOM	850	C	HIS	A	120	17.618	28.675	50.536	1.00	24.81	6

	ATOM	851	O	HIS	A	120	18.213	28.839	51.568	1.00	18.22	8
	ATOM	852	N	ILE	A	121	17.096	29.668	49.807	1.00	20.09	7
	ATOM	853	CD1	ILE	A	121	20.650	31.060	48.208	1.00	18.27	6
	ATOM	854	CG1	ILE	A	121	19.750	31.034	49.431	1.00	19.89	6
5	ATOM	855	CB	ILE	A	121	18.384	31.719	49.200	1.00	24.43	6
	ATOM	856	CG2	ILE	A	121	18.411	33.247	49.285	1.00	19.92	6
	ATOM	857	CA	ILE	A	121	17.296	31.108	50.101	1.00	27.30	6
	ATOM	858	C	ILE	A	121	15.996	31.862	49.892	1.00	18.34	6
	ATOM	859	O	ILE	A	121	15.345	31.498	48.913	1.00	21.09	8
10	ATOM	860	N	ILE	A	122	15.641	32.603	50.895	1.00	16.71	7
	ATOM	861	CD1	ILE	A	122	11.953	31.536	53.181	1.00	22.89	6
	ATOM	862	CG1	ILE	A	122	12.837	31.911	51.979	1.00	24.32	6
	ATOM	863	CB	ILE	A	122	13.522	33.267	52.001	1.00	22.36	6
	ATOM	864	CG2	ILE	A	122	12.472	34.387	52.058	1.00	22.28	6
15	ATOM	865	CA	ILE	A	122	14.414	33.410	50.792	1.00	17.89	6
	ATOM	866	C	ILE	A	122	14.873	34.891	50.714	1.00	20.53	6
	ATOM	867	O	ILE	A	122	15.632	35.335	51.596	1.00	18.10	8
	ATOM	868	N	ASN	A	123	14.457	35.638	49.735	1.00	24.14	7
	ATOM	869	ND2	ASN	A	123	14.634	39.722	47.933	1.00	17.66	7
20	ATOM	870	OD1	ASN	A	123	16.741	39.208	47.968	1.00	16.54	8
	ATOM	871	CG	ASN	A	123	15.601	38.839	48.002	1.00	18.32	6
	ATOM	872	CB	ASN	A	123	15.217	37.352	48.089	1.00	17.61	6
	ATOM	873	CA	ASN	A	123	14.771	37.063	49.516	1.00	16.49	6
	ATOM	874	C	ASN	A	123	13.519	37.846	49.924	1.00	16.16	6
25	ATOM	875	O	ASN	A	123	12.473	37.561	49.364	1.00	15.99	8
	ATOM	876	N	MET	A	124	13.682	38.631	51.003	1.00	17.91	7
	ATOM	877	CE	MET	A	124	12.625	37.065	55.122	1.00	18.43	6
	ATOM	878	SD	MET	A	124	10.961	37.279	54.473	1.00	25.22	16
	ATOM	879	CG	MET	A	124	11.393	37.747	52.785	1.00	22.98	6
30	ATOM	880	CB	MET	A	124	12.092	39.072	52.786	1.00	16.34	6
	ATOM	881	CA	MET	A	124	12.517	39.473	51.413	1.00	19.71	6
	ATOM	882	C	MET	A	124	12.848	40.994	51.279	1.00	22.29	6
	ATOM	883	O	MET	A	124	13.425	41.612	52.209	1.00	17.93	8
	ATOM	884	N	SER	A	125	12.669	41.567	50.101	1.00	19.47	7
35	ATOM	885	OG	SER	A	125	14.523	42.940	48.182	1.00	18.33	8
	ATOM	886	CB	SER	A	125	13.198	43.275	48.457	1.00	15.97	6
	ATOM	887	CA	SER	A	125	12.942	43.032	49.909	1.00	18.46	6
	ATOM	888	C	SER	A	125	11.655	43.750	50.350	1.00	20.28	6
	ATOM	889	O	SER	A	125	10.902	44.316	49.570	1.00	19.39	8
40	ATOM	890	N	LEU	A	126	11.297	43.695	51.624	1.00	17.62	7
	ATOM	891	CD2	LEU	A	126	8.102	40.862	51.658	1.00	24.63	6
	ATOM	892	CD1	LEU	A	126	8.622	41.714	53.877	1.00	23.93	6
	ATOM	893	CG	LEU	A	126	8.997	41.757	52.422	1.00	25.53	6
	ATOM	894	CB	LEU	A	126	8.916	43.187	51.871	1.00	28.42	6
45	ATOM	895	CA	LEU	A	126	10.051	44.199	52.184	1.00	26.68	6
	ATOM	896	C	LEU	A	126	10.270	44.487	53.671	1.00	21.12	6
	ATOM	897	O	LEU	A	126	11.254	44.020	54.240	1.00	20.64	8
	ATOM	898	N	GLY	A	127	9.505	45.329	54.335	1.00	22.92	7
	ATOM	899	CA	GLY	A	127	9.794	45.637	55.735	1.00	23.96	6
50	ATOM	900	C	GLY	A	127	8.602	46.346	56.347	1.00	29.15	6
	ATOM	901	O	GLY	A	127	7.718	46.926	55.745	1.00	30.52	8
	ATOM	902	N	SER	A	128	8.499	46.244	57.635	1.00	22.96	7
	ATOM	903	OG	SER	A	128	5.648	45.725	59.563	1.00	44.80	8
	ATOM	904	CB	SER	A	128	6.579	45.564	58.544	1.00	31.06	6
55	ATOM	905	CA	SER	A	128	7.422	46.809	58.423	1.00	26.75	6
	ATOM	906	C	SER	A	128	8.089	47.306	59.704	1.00	29.54	6
	ATOM	907	O	SER	A	128	9.118	46.792	60.156	1.00	25.89	8
	ATOM	908	N	THR	A	129	7.438	48.299	60.299	1.00	33.31	7
	ATOM	909	CG2	THR	A	129	7.743	51.258	60.493	1.00	30.94	6
60	ATOM	910	OG1	THR	A	129	6.191	50.069	61.840	1.00	40.54	8
	ATOM	911	CB	THR	A	129	7.555	50.360	61.680	1.00	32.41	6

350

	ATOM	912	CA	THR A 129	8.018	48.915	61.506	1.00	32.74	6
	ATOM	913	C	THR A 129	7.714	48.005	62.673	1.00	32.62	6
	ATOM	914	O	THR A 129	8.427	48.117	63.667	1.00	36.81	8
	ATOM	915	N	SER A 130	6.757	47.138	62.480	1.00	30.40	7
5	ATOM	916	OG	SER A 130	4.251	46.613	62.921	1.00	60.10	8
	ATOM	917	CB	SER A 130	5.130	46.585	64.070	1.00	57.43	6
	ATOM	918	CA	SER A 130	6.491	46.151	63.545	1.00	33.34	6
	ATOM	919	C	SER A 130	6.372	44.754	62.914	1.00	41.55	6
	ATOM	920	O	SER A 130	6.086	44.558	61.706	1.00	40.64	8
10	ATOM	921	N	GLY A 131	6.541	43.773	63.782	1.00	36.39	7
	ATOM	922	CA	GLY A 131	6.503	42.373	63.329	1.00	34.64	6
	ATOM	923	C	GLY A 131	5.234	41.724	63.822	1.00	35.04	6
	ATOM	924	O	GLY A 131	4.273	42.468	64.031	1.00	42.75	8
	ATOM	925	N	SER A 132	5.179	40.422	63.893	1.00	37.30	7
15	ATOM	926	OG	SER A 132	3.196	38.497	62.627	1.00	39.73	8
	ATOM	927	CB	SER A 132	2.876	39.643	63.376	1.00	35.53	6
	ATOM	928	CA	SER A 132	3.986	39.723	64.382	1.00	30.05	6
	ATOM	929	C	SER A 132	4.556	38.374	64.813	1.00	31.11	6
	ATOM	930	O	SER A 132	5.572	37.836	64.411	1.00	32.00	8
20	ATOM	931	N	SER A 133	3.842	37.734	65.695	1.00	32.96	7
	ATOM	932	OG	SER A 133	2.307	36.218	67.376	1.00	54.62	8
	ATOM	933	CB	SER A 133	3.700	36.342	67.576	1.00	47.70	6
	ATOM	934	CA	SER A 133	4.331	36.440	66.195	1.00	35.90	6
	ATOM	935	C	SER A 133	4.149	35.380	65.111	1.00	39.43	6
25	ATOM	936	O	SER A 133	4.847	34.366	65.010	1.00	33.00	8
	ATOM	937	N	THR A 134	3.180	35.667	64.251	1.00	37.16	7
	ATOM	938	CG2	THR A 134	1.470	34.464	61.014	1.00	42.89	6
	ATOM	939	OG1	THR A 134	0.694	35.406	63.113	1.00	55.08	8
	ATOM	940	CB	THR A 134	1.813	35.282	62.246	1.00	54.29	6
30	ATOM	941	CA	THR A 134	2.940	34.724	63.144	1.00	39.11	6
	ATOM	942	C	THR A 134	4.213	34.729	62.288	1.00	34.90	6
	ATOM	943	O	THR A 134	4.693	33.638	61.945	1.00	31.77	8
	ATOM	944	N	LEU A 135	4.600	35.994	62.058	1.00	30.88	7
	ATOM	945	CD2	LEU A 135	7.189	39.568	59.758	1.00	28.02	6
35	ATOM	946	CD1	LEU A 135	7.086	37.378	58.627	1.00	30.72	6
	ATOM	947	CG	LEU A 135	7.166	38.073	59.953	1.00	28.29	6
	ATOM	948	CB	LEU A 135	5.946	37.672	60.799	1.00	30.19	6
	ATOM	949	CA	LEU A 135	5.796	36.201	61.203	1.00	29.37	6
	ATOM	950	C	LEU A 135	7.077	35.635	61.777	1.00	27.29	6
40	ATOM	951	O	LEU A 135	7.958	35.025	61.154	1.00	28.89	8
	ATOM	952	N	GLU A 136	7.230	35.860	63.081	1.00	28.97	7
	ATOM	953	OE2	GLU A 136	8.946	36.631	67.630	1.00	43.17	8
	ATOM	954	OE1	GLU A 136	11.229	36.190	67.562	1.00	56.47	8
	ATOM	955	CD	GLU A 136	10.073	36.210	67.125	1.00	51.60	6
45	ATOM	956	CG	GLU A 136	9.871	35.664	65.729	1.00	37.60	6
	ATOM	957	CB	GLU A 136	8.518	35.957	65.118	1.00	30.71	6
	ATOM	958	CA	GLU A 136	8.494	35.399	63.696	1.00	25.88	6
	ATOM	959	C	GLU A 136	8.483	33.887	63.747	1.00	25.04	6
	ATOM	960	O	GLU A 136	9.527	33.244	63.636	1.00	29.33	8
50	ATOM	961	N	LEU A 137	7.287	33.373	64.028	1.00	26.02	7
	ATOM	962	CD2	LEU A 137	4.017	29.845	64.773	1.00	54.85	6
	ATOM	963	CD1	LEU A 137	6.341	29.084	65.226	1.00	54.59	6
	ATOM	964	CG	LEU A 137	5.487	30.026	64.399	1.00	53.18	6
	ATOM	965	CB	LEU A 137	5.909	31.481	64.461	1.00	42.31	6
55	ATOM	966	CA	LEU A 137	7.330	31.886	64.051	1.00	29.41	6
	ATOM	967	C	LEU A 137	7.745	31.326	62.696	1.00	31.98	6
	ATOM	968	O	LEU A 137	8.491	30.301	62.593	1.00	32.69	8
	ATOM	969	N	ALA A 138	7.170	31.984	61.676	1.00	27.96	7
	ATOM	970	CB	ALA A 138	6.457	32.131	59.296	1.00	25.09	6
60	ATOM	971	CA	ALA A 138	7.450	31.547	60.295	1.00	24.67	6
	ATOM	972	C	ALA A 138	8.946	31.641	60.041	1.00	26.68	6

351

	ATOM	973	O	ALA	A	138	9.651	30.736	59.482	1.00	24.43	8
	ATOM	974	N	VAL	A	139	9.509	32.777	60.481	1.00	24.43	7
	ATOM	975	CG2	VAL	A	139	10.805	35.468	59.644	1.00	23.18	6
	ATOM	976	CG1	VAL	A	139	12.736	34.458	60.955	1.00	25.26	6
5	ATOM	977	CB	VAL	A	139	11.240	34.427	60.639	1.00	23.72	6
	ATOM	978	CA	VAL	A	139	10.946	32.963	60.179	1.00	24.64	6
	ATOM	979	C	VAL	A	139	11.785	31.875	60.847	1.00	22.27	6
	ATOM	980	O	VAL	A	139	12.734	31.316	60.296	1.00	24.72	8
	ATOM	981	N	ASN	A	140	11.486	31.593	62.118	1.00	27.66	7
10	ATOM	982	ND2	ASN	A	140	11.683	32.285	66.008	1.00	42.32	7
	ATOM	983	OD1	ASN	A	140	13.425	32.414	64.611	1.00	36.78	8
	ATOM	984	CG	ASN	A	140	12.388	31.851	64.974	1.00	40.71	6
	ATOM	985	CB	ASN	A	140	11.762	30.648	64.308	1.00	38.24	6
	ATOM	986	CA	ASN	A	140	12.215	30.570	62.870	1.00	28.09	6
15	ATOM	987	C	ASN	A	140	12.048	29.142	62.314	1.00	23.74	6
	ATOM	988	O	ASN	A	140	13.079	28.438	62.234	1.00	27.56	8
	ATOM	989	N	ARG	A	141	10.819	28.818	61.934	1.00	29.30	7
	ATOM	990	NH2	ARG	A	141	6.667	24.020	60.976	1.00	62.35	7
	ATOM	991	NH1	ARG	A	141	7.366	25.245	59.341	1.00	62.64	7
20	ATOM	992	CZ	ARG	A	141	6.619	25.314	60.452	1.00	61.47	6
	ATOM	993	NE	ARG	A	141	6.129	26.266	61.285	1.00	59.61	7
	ATOM	994	CD	ARG	A	141	6.849	27.392	61.861	1.00	48.63	6
	ATOM	995	CG	ARG	A	141	8.296	26.951	62.044	1.00	33.19	6
	ATOM	996	CB	ARG	A	141	9.203	27.214	60.872	1.00	26.71	6
25	ATOM	997	CA	ARG	A	141	10.629	27.489	61.338	1.00	24.61	6
	ATOM	998	C	ARG	A	141	11.475	27.428	60.116	1.00	28.36	6
	ATOM	999	O	ARG	A	141	12.111	26.409	59.919	1.00	30.57	8
	ATOM	1000	N	ALA	A	142	11.510	28.420	59.220	1.00	28.76	7
	ATOM	1001	CB	ALA	A	142	12.125	29.617	57.121	1.00	22.79	6
30	ATOM	1002	CA	ALA	A	142	12.326	28.336	57.992	1.00	22.45	6
	ATOM	1003	C	ALA	A	142	13.799	28.193	58.312	1.00	23.46	6
	ATOM	1004	O	ALA	A	142	14.580	27.473	57.674	1.00	26.21	8
	ATOM	1005	N	ASN	A	143	14.220	28.995	59.297	1.00	27.87	7
	ATOM	1006	ND2	ASN	A	143	17.784	30.625	61.839	1.00	41.96	7
35	ATOM	1007	OD1	ASN	A	143	18.187	30.679	59.745	1.00	34.24	8
	ATOM	1008	CG	ASN	A	143	17.322	30.588	60.596	1.00	29.11	6
	ATOM	1009	CB	ASN	A	143	15.871	30.329	60.523	1.00	29.69	6
	ATOM	1010	CA	ASN	A	143	15.635	29.021	59.743	1.00	30.16	6
	ATOM	1011	C	ASN	A	143	15.953	27.666	60.335	1.00	30.12	6
40	ATOM	1012	O	ASN	A	143	17.010	27.136	59.946	1.00	31.87	8
	ATOM	1013	N	ASN	A	144	15.008	27.125	61.112	1.00	29.34	7
	ATOM	1014	ND2	ASN	A	144	15.977	26.890	64.048	1.00	45.86	7
	ATOM	1015	OD1	ASN	A	144	13.874	26.581	64.829	1.00	57.91	8
	ATOM	1016	CG	ASN	A	144	14.771	26.309	63.974	1.00	57.11	6
45	ATOM	1017	CB	ASN	A	144	14.450	25.359	62.806	1.00	44.00	6
	ATOM	1018	CA	ASN	A	144	15.299	25.781	61.618	1.00	29.75	6
	ATOM	1019	C	ASN	A	144	15.282	24.762	60.497	1.00	40.41	6
	ATOM	1020	O	ASN	A	144	15.968	23.716	60.573	1.00	42.44	8
	ATOM	1021	N	ALA	A	145	14.528	25.050	59.457	1.00	34.04	7
50	ATOM	1022	CB	ALA	A	145	13.330	24.281	57.390	1.00	26.85	6
	ATOM	1023	CA	ALA	A	145	14.483	24.121	58.327	1.00	20.42	6
	ATOM	1024	C	ALA	A	145	15.731	24.288	57.552	1.00	23.85	6
	ATOM	1025	O	ALA	A	145	15.664	23.663	56.514	1.00	30.91	8
	ATOM	1026	N	GLY	A	146	16.740	25.040	57.840	1.00	26.51	7
55	ATOM	1027	CA	GLY	A	146	17.921	25.100	56.958	1.00	22.88	6
	ATOM	1028	C	GLY	A	146	17.767	26.214	55.904	1.00	27.41	6
	ATOM	1029	O	GLY	A	146	18.735	26.130	55.122	1.00	24.39	8
	ATOM	1030	N	ILE	A	147	16.707	27.049	55.889	1.00	21.34	7
	ATOM	1031	CD1	ILE	A	147	13.320	27.096	53.722	1.00	23.01	6
60	ATOM	1032	CG1	ILE	A	147	14.789	27.060	54.041	1.00	22.99	6
	ATOM	1033	CB	ILE	A	147	15.321	28.439	54.332	1.00	26.62	6

352

	ATOM	1034	CG2	ILE	A	147	15.232	29.384	53.135	1.00	23.14	6
	ATOM	1035	CA	ILE	A	147	16.730	28.111	54.845	1.00	26.00	6
	ATOM	1036	C	ILE	A	147	17.500	29.398	55.235	1.00	18.99	6
	ATOM	1037	O	ILE	A	147	17.385	29.727	56.411	1.00	20.20	8
5	ATOM	1038	N	LEU	A	148	18.230	30.007	54.320	1.00	20.50	7
	ATOM	1039	CD2	LEU	A	148	21.996	32.963	53.094	1.00	21.59	6
	ATOM	1040	CD1	LEU	A	148	21.187	32.871	55.483	1.00	21.52	6
	ATOM	1041	CG	LEU	A	148	20.849	32.729	54.004	1.00	21.01	6
	ATOM	1042	CB	LEU	A	148	20.076	31.416	53.699	1.00	21.28	6
10	ATOM	1043	CA	LEU	A	148	18.874	31.288	54.622	1.00	18.16	6
	ATOM	1044	C	LEU	A	148	17.890	32.403	54.204	1.00	21.69	6
	ATOM	1045	O	LEU	A	148	17.385	32.443	53.053	1.00	18.87	8
	ATOM	1046	N	LEU	A	149	17.504	33.244	55.115	1.00	19.79	7
	ATOM	1047	CD2	LEU	A	149	13.039	33.698	56.361	1.00	21.21	6
15	ATOM	1048	CD1	LEU	A	149	14.937	32.303	57.044	1.00	29.79	6
	ATOM	1049	CG	LEU	A	149	14.430	33.273	55.986	1.00	23.63	6
	ATOM	1050	CB	LEU	A	149	15.412	34.443	55.914	1.00	19.13	6
	ATOM	1051	CA	LEU	A	149	16.580	34.382	54.989	1.00	18.47	6
	ATOM	1052	C	LEU	A	149	17.403	35.669	54.993	1.00	22.25	6
20	ATOM	1053	O	LEU	A	149	18.294	35.913	55.802	1.00	19.26	8
	ATOM	1054	N	VAL	A	150	17.140	36.501	53.974	1.00	21.30	7
	ATOM	1055	CG2	VAL	A	150	19.747	36.476	52.518	1.00	19.59	6
	ATOM	1056	CG1	VAL	A	150	19.570	38.785	52.177	1.00	22.93	6
	ATOM	1057	CB	VAL	A	150	18.710	37.578	52.402	1.00	20.01	6
25	ATOM	1058	CA	VAL	A	150	17.846	37.764	53.660	1.00	20.55	6
	ATOM	1059	C	VAL	A	150	16.751	38.844	53.547	1.00	18.11	6
	ATOM	1060	O	VAL	A	150	15.817	38.657	52.756	1.00	18.48	8
	ATOM	1061	N	GLY	A	151	16.896	39.886	54.338	1.00	16.89	7
	ATOM	1062	CA	GLY	A	151	15.849	40.980	54.289	1.00	20.73	6
30	ATOM	1063	C	GLY	A	151	16.402	42.404	54.347	1.00	16.63	6
	ATOM	1064	O	GLY	A	151	17.563	42.678	54.734	1.00	16.14	8
	ATOM	1065	N	ALA	A	152	15.614	43.322	53.807	1.00	17.20	7
	ATOM	1066	CB	ALA	A	152	14.900	45.297	52.755	1.00	14.94	6
	ATOM	1067	CA	ALA	A	152	15.998	44.737	53.682	1.00	14.71	6
35	ATOM	1068	C	ALA	A	152	15.895	45.381	55.071	1.00	13.99	6
	ATOM	1069	O	ALA	A	152	14.892	45.173	55.788	1.00	17.68	8
	ATOM	1070	N	ALA	A	153	16.952	46.133	55.387	1.00	16.31	7
	ATOM	1071	CB	ALA	A	153	18.293	47.552	56.901	1.00	17.15	6
	ATOM	1072	CA	ALA	A	153	16.956	46.875	56.681	1.00	16.19	6
40	ATOM	1073	C	ALA	A	153	15.860	47.945	56.800	1.00	22.55	6
	ATOM	1074	O	ALA	A	153	15.313	48.113	57.913	1.00	22.09	8
	ATOM	1075	N	GLY	A	154	15.484	48.543	55.690	1.00	16.09	7
	ATOM	1076	CA	GLY	A	154	14.427	49.555	55.683	1.00	18.21	6
	ATOM	1077	C	GLY	A	154	15.049	50.809	55.066	1.00	14.46	6
45	ATOM	1078	O	GLY	A	154	16.263	50.930	54.899	1.00	16.40	8
	ATOM	1079	N	ASN	A	155	14.113	51.674	54.663	1.00	20.62	7
	ATOM	1080	ND2	ASN	A	155	13.511	51.960	50.428	1.00	16.52	7
	ATOM	1081	OD1	ASN	A	155	15.360	51.538	51.718	1.00	19.81	8
	ATOM	1082	CG	ASN	A	155	14.233	52.033	51.537	1.00	17.87	6
50	ATOM	1083	CB	ASN	A	155	13.765	52.902	52.677	1.00	18.24	6
	ATOM	1084	CA	ASN	A	155	14.551	52.936	53.989	1.00	17.90	6
	ATOM	1085	C	ASN	A	155	14.159	54.123	54.891	1.00	24.83	6
	ATOM	1086	O	ASN	A	155	13.733	55.098	54.292	1.00	22.47	8
	ATOM	1087	N	THR	A	156	14.154	53.978	56.193	1.00	20.39	7
55	ATOM	1088	CG2	THR	A	156	12.287	53.113	58.276	1.00	23.08	6
	ATOM	1089	OG1	THR	A	156	14.307	54.076	59.118	1.00	23.01	8
	ATOM	1090	CB	THR	A	156	13.124	54.367	58.402	1.00	23.69	6
	ATOM	1091	CA	THR	A	156	13.714	54.997	57.116	1.00	24.79	6
	ATOM	1092	C	THR	A	156	14.848	56.011	57.320	1.00	29.93	6
60	ATOM	1093	O	THR	A	156	14.402	57.042	57.813	1.00	27.99	8
	ATOM	1094	N	GLY	A	157	16.086	55.856	57.005	1.00	20.16	7

353

	ATOM	1095	CA	GLY	A	157	17.154	56.785	57.245	1.00	25.10	6
	ATOM	1096	C	GLY	A	157	17.486	57.000	58.723	1.00	29.14	6
	ATOM	1097	O	GLY	A	157	18.377	57.810	58.961	1.00	33.04	8
	ATOM	1098	N	ARG	A	160	16.904	56.334	59.657	1.00	25.62	7
5	ATOM	1099	NH2	ARG	A	160	10.330	58.682	62.645	1.00	60.34	7
	ATOM	1100	NH1	ARG	A	160	12.170	59.527	63.732	1.00	59.53	7
	ATOM	1101	CZ	ARG	A	160	11.711	58.643	62.754	1.00	59.28	6
	ATOM	1102	NE	ARG	A	160	12.583	57.864	61.970	1.00	57.95	7
	ATOM	1103	CD	ARG	A	160	13.994	58.266	62.165	1.00	51.11	6
10	ATOM	1104	CG	ARG	A	160	15.060	57.898	61.220	1.00	42.14	6
	ATOM	1105	CB	ARG	A	160	15.570	56.502	61.634	1.00	31.02	6
	ATOM	1106	CA	ARG	A	160	17.041	56.392	61.112	1.00	28.11	6
	ATOM	1107	C	ARG	A	160	17.381	55.048	61.710	1.00	30.03	6
	ATOM	1108	O	ARG	A	160	17.398	54.049	60.983	1.00	27.84	8
15	ATOM	1109	N	GLN	A	161	17.535	55.017	63.000	1.00	26.77	7
	ATOM	1110	NE2	GLN	A	161	19.350	52.013	67.864	1.00	60.74	7
	ATOM	1111	OE1	GLN	A	161	20.262	53.735	66.798	1.00	59.57	8
	ATOM	1112	CD	GLN	A	161	19.355	52.904	66.883	1.00	58.69	6
	ATOM	1113	CG	GLN	A	161	18.232	52.759	65.891	1.00	34.77	6
20	ATOM	1114	CB	GLN	A	161	18.519	53.945	64.970	1.00	30.48	6
	ATOM	1115	CA	GLN	A	161	17.801	53.757	63.664	1.00	23.33	6
	ATOM	1116	C	GLN	A	161	16.520	52.971	63.833	1.00	29.67	6
	ATOM	1117	O	GLN	A	161	15.474	53.589	63.955	1.00	29.09	8
	ATOM	1118	N	GLY	A	162	16.517	51.663	63.859	1.00	24.53	7
25	ATOM	1119	CA	GLY	A	162	15.351	50.793	64.031	1.00	20.53	6
	ATOM	1120	C	GLY	A	162	15.104	49.941	62.796	1.00	26.19	6
	ATOM	1121	O	GLY	A	162	14.288	50.249	61.907	1.00	22.33	8
	ATOM	1122	N	VAL	A	165	15.844	48.832	62.774	1.00	25.37	7
	ATOM	1123	CG2	VAL	A	165	18.242	47.376	61.823	1.00	20.11	6
30	ATOM	1124	CG1	VAL	A	165	16.767	45.785	60.528	1.00	21.35	6
	ATOM	1125	CB	VAL	A	165	16.841	46.808	61.703	1.00	22.43	6
	ATOM	1126	CA	VAL	A	165	15.776	47.891	61.618	1.00	20.88	6
	ATOM	1127	C	VAL	A	165	14.383	47.384	61.360	1.00	24.44	6
	ATOM	1128	O	VAL	A	165	13.793	46.948	62.359	1.00	22.51	8
35	ATOM	1129	N	ASN	A	166	13.847	47.458	60.151	1.00	20.59	7
	ATOM	1130	ND2	ASN	A	166	11.804	49.622	59.063	1.00	37.01	7
	ATOM	1131	OD1	ASN	A	166	11.291	48.862	57.045	1.00	40.47	8
	ATOM	1132	CG	ASN	A	166	11.691	48.612	58.213	1.00	36.75	6
	ATOM	1133	CB	ASN	A	166	12.084	47.201	58.564	1.00	18.42	6
40	ATOM	1134	CA	ASN	A	166	12.480	46.925	60.012	1.00	20.41	6
	ATOM	1135	C	ASN	A	166	12.430	45.397	60.220	1.00	27.19	6
	ATOM	1136	O	ASN	A	166	13.394	44.641	60.213	1.00	20.29	8
	ATOM	1137	N	TYR	A	167	11.219	44.939	60.323	1.00	23.03	7
	ATOM	1138	OH	TYR	A	167	10.922	44.540	66.485	1.00	45.50	8
45	ATOM	1139	CD2	TYR	A	167	9.715	44.838	63.205	1.00	34.30	6
	ATOM	1140	CE2	TYR	A	167	10.084	45.141	64.501	1.00	27.99	6
	ATOM	1141	CZ	TYR	A	167	10.625	44.092	65.233	1.00	48.07	6
	ATOM	1142	CE1	TYR	A	167	10.871	42.802	64.754	1.00	30.09	6
	ATOM	1143	CD1	TYR	A	167	10.582	42.588	63.401	1.00	27.59	6
50	ATOM	1144	CG	TYR	A	167	9.959	43.576	62.657	1.00	30.84	6
	ATOM	1145	CB	TYR	A	167	9.537	43.461	61.197	1.00	25.40	6
	ATOM	1146	CA	TYR	A	167	10.830	43.562	60.383	1.00	22.25	6
	ATOM	1147	C	TYR	A	167	10.479	43.048	58.968	1.00	26.82	6
	ATOM	1148	O	TYR	A	167	9.785	43.740	58.230	1.00	28.17	8
55	ATOM	1149	N	PRO	A	168	10.803	41.830	58.559	1.00	24.12	7
	ATOM	1150	CG	PRO	A	168	11.069	39.952	57.192	1.00	21.03	6
	ATOM	1151	CD	PRO	A	168	10.376	41.337	57.220	1.00	18.66	6
	ATOM	1152	CB	PRO	A	168	11.014	39.509	58.639	1.00	20.70	6
	ATOM	1153	CA	PRO	A	168	11.468	40.788	59.357	1.00	21.08	6
60	ATOM	1154	C	PRO	A	168	12.960	40.862	59.456	1.00	22.02	6
	ATOM	1155	O	PRO	A	168	13.492	39.981	60.180	1.00	21.94	8

354

	ATOM	1156	N	ALA	A	169	13.657	41.831	58.841	1.00	15.90	7
	ATOM	1157	CB	ALA	A	169	15.736	42.908	58.091	1.00	17.37	6
	ATOM	1158	CA	ALA	A	169	15.106	41.851	58.949	1.00	15.97	6
	ATOM	1159	C	ALA	A	169	15.607	41.947	60.374	1.00	21.06	6
5	ATOM	1160	O	ALA	A	169	16.752	41.565	60.663	1.00	21.07	8
	ATOM	1161	N	ARG	A	170	14.833	42.498	61.289	1.00	21.46	7
	ATOM	1162	NH2	ARG	A	170	13.387	47.123	67.747	1.00	60.78	7
	ATOM	1163	NH1	ARG	A	170	13.043	47.610	65.444	1.00	49.63	7
	ATOM	1164	CZ	ARG	A	170	13.604	46.896	66.440	1.00	59.33	6
10	ATOM	1165	NE	ARG	A	170	14.377	45.776	66.226	1.00	56.52	7
	ATOM	1166	CD	ARG	A	170	14.143	45.240	64.921	1.00	35.28	6
	ATOM	1167	CG	ARG	A	170	15.134	44.173	64.633	1.00	26.86	6
	ATOM	1168	CB	ARG	A	170	14.382	43.573	63.430	1.00	22.20	6
	ATOM	1169	CA	ARG	A	170	15.339	42.683	62.653	1.00	22.58	6
15	ATOM	1170	C	ARG	A	170	15.423	41.335	63.390	1.00	26.44	6
	ATOM	1171	O	ARG	A	170	16.298	41.175	64.268	1.00	24.76	8
	ATOM	1172	N	TYR	A	171	14.601	40.421	63.006	1.00	21.88	7
	ATOM	1173	OH	TYR	A	171	8.238	39.587	63.993	1.00	30.27	8
	ATOM	1174	CD2	TYR	A	171	11.260	38.551	62.366	1.00	23.79	6
20	ATOM	1175	CE2	TYR	A	171	9.930	38.895	62.534	1.00	26.08	6
	ATOM	1176	CZ	TYR	A	171	9.544	39.258	63.827	1.00	22.66	6
	ATOM	1177	CE1	TYR	A	171	10.437	39.256	64.849	1.00	22.42	6
	ATOM	1178	CD1	TYR	A	171	11.754	38.908	64.657	1.00	25.00	6
	ATOM	1179	CG	TYR	A	171	12.190	38.520	63.397	1.00	20.82	6
25	ATOM	1180	CB	TYR	A	171	13.614	38.157	63.120	1.00	20.76	6
	ATOM	1181	CA	TYR	A	171	14.662	39.115	63.666	1.00	21.12	6
	ATOM	1182	C	TYR	A	171	16.019	38.496	63.429	1.00	23.66	6
	ATOM	1183	O	TYR	A	171	16.595	38.612	62.377	1.00	19.53	8
	ATOM	1184	N	SER	A	172	16.590	37.805	64.409	1.00	21.25	7
30	ATOM	1185	OG	SER	A	172	18.439	36.736	66.290	1.00	40.47	8
	ATOM	1186	CB	SER	A	172	17.643	36.002	65.430	1.00	30.04	6
	ATOM	1187	CA	SER	A	172	17.855	37.114	64.343	1.00	18.01	6
	ATOM	1188	C	SER	A	172	17.972	36.098	63.241	1.00	18.07	6
	ATOM	1189	O	SER	A	172	19.076	35.857	62.794	1.00	24.44	8
35	ATOM	1190	N	GLY	A	173	16.849	35.497	62.953	1.00	20.49	7
	ATOM	1191	CA	GLY	A	173	16.895	34.520	61.849	1.00	25.76	6
	ATOM	1192	C	GLY	A	173	17.065	35.136	60.466	1.00	28.21	6
	ATOM	1193	O	GLY	A	173	17.142	34.299	59.561	1.00	24.65	8
	ATOM	1194	N	VAL	A	174	17.037	36.454	60.298	1.00	22.37	7
40	ATOM	1195	CG2	VAL	A	174	14.544	37.187	59.094	1.00	21.22	6
	ATOM	1196	CG1	VAL	A	174	15.868	38.353	57.251	1.00	16.19	6
	ATOM	1197	CB	VAL	A	174	15.853	37.860	58.711	1.00	19.69	6
	ATOM	1198	CA	VAL	A	174	17.081	37.002	58.950	1.00	18.39	6
	ATOM	1199	C	VAL	A	174	18.302	37.804	58.794	1.00	20.62	6
45	ATOM	1200	O	VAL	A	174	18.537	38.493	59.767	1.00	20.79	8
	ATOM	1201	N	MET	A	175	19.071	37.843	57.763	1.00	19.50	7
	ATOM	1202	CE	MET	A	175	24.752	37.650	55.722	1.00	18.78	6
	ATOM	1203	SD	MET	A	175	23.178	36.899	55.344	1.00	28.69	16
	ATOM	1204	CG	MET	A	175	22.276	37.473	56.764	1.00	30.99	6
50	ATOM	1205	CB	MET	A	175	21.073	38.209	56.458	1.00	18.24	6
	ATOM	1206	CA	MET	A	175	20.269	38.719	57.673	1.00	19.14	6
	ATOM	1207	C	MET	A	175	19.808	40.085	57.181	1.00	19.60	6
	ATOM	1208	O	MET	A	175	19.243	40.124	56.075	1.00	19.68	8
	ATOM	1209	N	ALA	A	176	19.998	41.141	57.911	1.00	20.50	7
55	ATOM	1210	CB	ALA	A	176	19.374	43.342	58.912	1.00	17.16	6
	ATOM	1211	CA	ALA	A	176	19.559	42.523	57.638	1.00	18.51	6
	ATOM	1212	C	ALA	A	176	20.608	43.146	56.758	1.00	15.58	6
	ATOM	1213	O	ALA	A	176	21.802	43.226	57.028	1.00	18.20	8
	ATOM	1214	N	VAL	A	177	20.119	43.546	55.592	1.00	17.19	7
60	ATOM	1215	CG2	VAL	A	177	20.837	41.990	53.335	1.00	16.29	6
	ATOM	1216	CG1	VAL	A	177	21.783	44.025	52.249	1.00	12.13	6

355

	ATOM	1217	CB	VAL	A	177	20.739	43.505	53.233	1.00	14.48	6
	ATOM	1218	CA	VAL	A	177	21.011	44.188	54.618	1.00	18.22	6
	ATOM	1219	C	VAL	A	177	20.828	45.734	54.489	1.00	19.81	6
	ATOM	1220	O	VAL	A	177	19.728	46.259	54.253	1.00	16.83	8
5	ATOM	1221	N	ALA	A	178	21.957	46.444	54.565	1.00	17.27	7
	ATOM	1222	CB	ALA	A	178	23.054	48.386	55.452	1.00	14.79	6
	ATOM	1223	CA	ALA	A	178	22.035	47.894	54.418	1.00	18.82	6
	ATOM	1224	C	ALA	A	178	22.445	48.215	52.970	1.00	17.05	6
	ATOM	1225	O	ALA	A	178	23.095	47.447	52.260	1.00	16.34	8
10	ATOM	1226	N	ALA	A	179	22.014	49.381	52.483	1.00	18.05	7
	ATOM	1227	CB	ALA	A	179	21.168	50.710	50.548	1.00	14.34	6
	ATOM	1228	CA	ALA	A	179	22.317	49.940	51.148	1.00	17.14	6
	ATOM	1229	C	ALA	A	179	23.496	50.901	51.162	1.00	16.16	6
	ATOM	1230	O	ALA	A	179	23.525	51.777	52.044	1.00	18.65	8
15	ATOM	1231	N	VAL	A	180	24.451	50.812	50.317	1.00	14.26	7
	ATOM	1232	CG2	VAL	A	180	27.438	49.981	49.469	1.00	17.76	6
	ATOM	1233	CG1	VAL	A	180	26.913	50.487	51.890	1.00	16.51	6
	ATOM	1234	CB	VAL	A	180	26.964	50.989	50.462	1.00	17.24	6
	ATOM	1235	CA	VAL	A	180	25.609	51.616	50.075	1.00	17.31	6
20	ATOM	1236	C	VAL	A	180	25.586	52.187	48.644	1.00	22.88	6
	ATOM	1237	O	VAL	A	180	24.947	51.671	47.675	1.00	20.50	8
	ATOM	1238	N	ASP	A	181	26.291	53.321	48.446	1.00	25.29	7
	ATOM	1239	OD2	ASP	A	181	27.098	57.308	48.607	1.00	32.67	8
	ATOM	1240	OD1	ASP	A	181	28.547	55.806	48.232	1.00	27.26	8
25	ATOM	1241	CG	ASP	A	181	27.399	56.184	48.028	1.00	26.15	6
	ATOM	1242	CB	ASP	A	181	26.254	55.570	47.285	1.00	24.05	6
	ATOM	1243	CA	ASP	A	181	26.408	54.054	47.131	1.00	22.55	6
	ATOM	1244	C	ASP	A	181	27.687	53.624	46.461	1.00	28.00	6
	ATOM	1245	O	ASP	A	181	28.393	52.695	46.923	1.00	23.27	8
30	ATOM	1246	N	GLN	A	182	28.038	54.220	45.348	1.00	23.99	7
	ATOM	1247	NE2	GLN	A	182	28.625	56.347	43.392	1.00	59.18	7
	ATOM	1248	OE1	GLN	A	182	26.424	55.625	43.700	1.00	51.62	8
	ATOM	1249	CD	GLN	A	182	27.579	55.498	43.211	1.00	57.87	6
	ATOM	1250	CG	GLN	A	182	28.188	54.342	42.400	1.00	36.22	6
35	ATOM	1251	CB	GLN	A	182	29.458	54.178	43.178	1.00	29.07	6
	ATOM	1252	CA	GLN	A	182	29.220	53.827	44.637	1.00	20.63	6
	ATOM	1253	C	GLN	A	182	30.498	54.185	45.347	1.00	22.86	6
	ATOM	1254	O	GLN	A	182	31.519	53.726	44.882	1.00	27.70	8
	ATOM	1255	N	ASN	A	183	30.450	54.957	46.362	1.00	27.14	7
40	ATOM	1256	ND2	ASN	A	183	30.750	58.651	46.572	1.00	47.15	7
	ATOM	1257	OD1	ASN	A	183	32.423	57.377	45.640	1.00	47.21	8
	ATOM	1258	CG	ASN	A	183	31.593	57.633	46.532	1.00	47.14	6
	ATOM	1259	CB	ASN	A	183	31.436	56.677	47.691	1.00	32.89	6
	ATOM	1260	CA	ASN	A	183	31.656	55.252	47.134	1.00	31.50	6
45	ATOM	1261	C	ASN	A	183	31.698	54.274	48.330	1.00	33.88	6
	ATOM	1262	O	ASN	A	183	32.459	54.594	49.245	1.00	32.01	8
	ATOM	1263	N	GLY	A	184	30.838	53.306	48.492	1.00	23.02	7
	ATOM	1264	CA	GLY	A	184	30.887	52.499	49.688	1.00	23.48	6
	ATOM	1265	C	GLY	A	184	30.322	53.209	50.879	1.00	26.52	6
50	ATOM	1266	O	GLY	A	184	30.461	52.723	52.013	1.00	30.46	8
	ATOM	1267	N	GLN	A	185	29.568	54.273	50.751	1.00	27.03	7
	ATOM	1268	NE2	GLN	A	185	30.258	58.823	51.467	1.00	60.06	7
	ATOM	1269	OE1	GLN	A	185	31.633	57.570	53.078	1.00	61.27	8
	ATOM	1270	CD	GLN	A	185	30.896	57.806	52.089	1.00	58.99	6
55	ATOM	1271	CG	GLN	A	185	30.465	56.526	51.381	1.00	54.79	6
	ATOM	1272	CB	GLN	A	185	29.023	56.422	51.884	1.00	24.50	6
	ATOM	1273	CA	GLN	A	185	29.012	54.889	51.969	1.00	24.00	6
	ATOM	1274	C	GLN	A	185	27.518	54.587	52.026	1.00	20.62	6
	ATOM	1275	O	GLN	A	185	26.870	54.488	51.012	1.00	21.50	8
60	ATOM	1276	N	ARG	A	186	27.110	54.610	53.277	1.00	20.28	7
	ATOM	1277	NH2	ARG	A	186	21.131	56.372	57.060	1.00	27.55	7

356

	ATOM	1278	NH1	ARG	A	186	22.904	57.626	57.258	1.00	35.29	7
	ATOM	1279	CZ	ARG	A	186	22.478	56.400	57.225	1.00	38.65	6
	ATOM	1280	NE	ARG	A	186	23.030	55.217	57.167	1.00	30.56	7
	ATOM	1281	CD	ARG	A	186	24.081	54.434	56.803	1.00	26.76	6
5	ATOM	1282	CG	ARG	A	186	24.037	54.280	55.356	1.00	19.72	6
	ATOM	1283	CB	ARG	A	186	25.484	54.482	55.024	1.00	17.77	6
	ATOM	1284	CA	ARG	A	186	25.719	54.343	53.529	1.00	19.83	6
	ATOM	1285	C	ARG	A	186	24.849	55.249	52.697	1.00	29.56	6
	ATOM	1286	O	ARG	A	186	25.067	56.444	52.788	1.00	26.52	8
10	ATOM	1287	N	ALA	A	187	23.822	54.843	52.015	1.00	19.87	7
	ATOM	1288	CB	ALA	A	187	22.098	54.655	50.429	1.00	22.61	6
	ATOM	1289	CA	ALA	A	187	22.847	55.634	51.325	1.00	21.48	6
	ATOM	1290	C	ALA	A	187	22.107	56.312	52.498	1.00	23.68	6
	ATOM	1291	O	ALA	A	187	21.762	55.850	53.579	1.00	20.22	8
15	ATOM	1292	N	SER	A	188	21.706	57.586	52.332	1.00	22.67	7
	ATOM	1293	OG	SER	A	188	19.942	59.678	51.654	1.00	31.32	8
	ATOM	1294	CB	SER	A	188	20.789	59.773	52.799	1.00	27.54	6
	ATOM	1295	CA	SER	A	188	21.069	58.367	53.386	1.00	26.75	6
	ATOM	1296	C	SER	A	188	19.792	57.706	53.819	1.00	22.34	6
20	ATOM	1297	O	SER	A	188	19.413	58.002	54.966	1.00	22.84	8
	ATOM	1298	N	PHE	A	189	19.037	56.941	53.001	1.00	22.24	7
	ATOM	1299	CD2	PHE	A	189	17.852	56.033	49.969	1.00	17.59	6
	ATOM	1300	CE2	PHE	A	189	18.514	55.372	48.951	1.00	24.96	6
	ATOM	1301	CZ	PHE	A	189	18.791	54.030	49.053	1.00	21.12	6
25	ATOM	1302	CE1	PHE	A	189	18.335	53.422	50.233	1.00	19.91	6
	ATOM	1303	CD1	PHE	A	189	17.679	54.049	51.248	1.00	19.31	6
	ATOM	1304	CG	PHE	A	189	17.414	55.447	51.104	1.00	24.18	6
	ATOM	1305	CB	PHE	A	189	16.754	56.211	52.243	1.00	17.91	6
	ATOM	1306	CA	PHE	A	189	17.738	56.340	53.411	1.00	19.41	6
30	ATOM	1307	C	PHE	A	189	17.900	54.995	54.158	1.00	13.56	6
	ATOM	1308	O	PHE	A	189	16.915	54.531	54.699	1.00	19.77	8
	ATOM	1309	N	SER	A	190	19.127	54.513	54.121	1.00	17.30	7
	ATOM	1310	OG	SER	A	190	20.958	51.491	54.627	1.00	18.49	8
	ATOM	1311	CB	SER	A	190	20.614	52.728	54.152	1.00	19.55	6
35	ATOM	1312	CA	SER	A	190	19.310	53.198	54.732	1.00	19.59	6
	ATOM	1313	C	SER	A	190	19.165	53.145	56.233	1.00	19.58	6
	ATOM	1314	O	SER	A	190	19.993	53.714	56.959	1.00	22.91	8
	ATOM	1315	N	THR	A	191	18.230	52.366	56.775	1.00	20.03	7
	ATOM	1316	CG2	THR	A	191	16.453	50.951	59.871	1.00	19.20	6
40	ATOM	1317	OG1	THR	A	191	15.685	51.953	57.813	1.00	23.24	8
	ATOM	1318	CB	THR	A	191	16.775	51.284	58.421	1.00	18.08	6
	ATOM	1319	CA	THR	A	191	17.970	52.140	58.186	1.00	19.69	6
	ATOM	1320	C	THR	A	191	19.214	51.465	58.784	1.00	26.64	6
	ATOM	1321	O	THR	A	191	19.971	50.764	58.083	1.00	20.74	8
45	ATOM	1322	N	TYR	A	192	19.509	51.785	60.037	1.00	24.10	7
	ATOM	1323	OH	TYR	A	192	20.579	57.242	62.652	1.00	42.72	8
	ATOM	1324	CD2	TYR	A	192	21.008	54.515	60.307	1.00	27.03	6
	ATOM	1325	CE2	TYR	A	192	20.670	55.799	60.760	1.00	28.72	6
	ATOM	1326	CZ	TYR	A	192	20.864	56.031	62.103	1.00	37.26	6
50	ATOM	1327	CE1	TYR	A	192	21.348	55.083	63.015	1.00	36.10	6
	ATOM	1328	CD1	TYR	A	192	21.652	53.820	62.541	1.00	25.25	6
	ATOM	1329	CG	TYR	A	192	21.516	53.550	61.169	1.00	22.50	6
	ATOM	1330	CB	TYR	A	192	21.910	52.154	60.684	1.00	25.18	6
	ATOM	1331	CA	TYR	A	192	20.708	51.213	60.683	1.00	17.72	6
55	ATOM	1332	C	TYR	A	192	20.258	50.806	62.081	1.00	17.55	6
	ATOM	1333	O	TYR	A	192	19.128	50.985	62.559	1.00	19.29	8
	ATOM	1334	N	GLY	A	193	21.198	50.136	62.735	1.00	19.36	7
	ATOM	1335	CA	GLY	A	193	20.866	49.639	64.090	1.00	21.59	6
	ATOM	1336	C	GLY	A	193	21.817	48.449	64.262	1.00	23.46	6
60	ATOM	1337	O	GLY	A	193	22.550	48.074	63.361	1.00	19.67	8
	ATOM	1338	N	PRO	A	194	21.782	47.949	65.484	1.00	25.90	7

357

	ATOM	1339	CG	PRO A 194	20.970	47.337	67.684	1.00	27.38	6
	ATOM	1340	CD	PRO A 194	20.887	48.403	66.615	1.00	27.18	6
	ATOM	1341	CB	PRO A 194	22.239	46.658	67.360	1.00	22.45	6
	ATOM	1342	CA	PRO A 194	22.600	46.837	65.880	1.00	28.03	6
5	ATOM	1343	C	PRO A 194	22.412	45.568	65.036	1.00	21.43	6
	ATOM	1344	O	PRO A 194	23.318	44.731	64.998	1.00	22.19	8
	ATOM	1345	N	GLU A 195	21.274	45.405	64.424	1.00	20.23	7
	ATOM	1346	OE2	GLU A 195	18.569	46.243	65.075	1.00	24.87	8
	ATOM	1347	OE1	GLU A 195	17.965	44.957	66.720	1.00	35.26	8
10	ATOM	1348	CD	GLU A 195	18.409	45.076	65.595	1.00	28.08	6
	ATOM	1349	CG	GLU A 195	18.768	43.825	64.864	1.00	21.18	6
	ATOM	1350	CB	GLU A 195	19.456	43.945	63.541	1.00	16.96	6
	ATOM	1351	CA	GLU A 195	20.940	44.207	63.686	1.00	20.96	6
	ATOM	1352	C	GLU A 195	21.528	44.212	62.285	1.00	30.31	6
15	ATOM	1353	O	GLU A 195	21.450	43.118	61.697	1.00	23.68	8
	ATOM	1354	N	ILE A 196	22.053	45.362	61.843	1.00	19.60	7
	ATOM	1355	CD1	ILE A 196	20.930	47.604	59.167	1.00	17.78	6
	ATOM	1356	CG1	ILE A 196	22.141	47.811	60.018	1.00	16.83	6
	ATOM	1357	CB	ILE A 196	23.248	46.768	60.069	1.00	20.89	6
20	ATOM	1358	CG2	ILE A 196	23.876	46.679	58.658	1.00	16.59	6
	ATOM	1359	CA	ILE A 196	22.643	45.435	60.528	1.00	20.36	6
	ATOM	1360	C	ILE A 196	23.722	44.323	60.503	1.00	23.09	6
	ATOM	1361	O	ILE A 196	24.633	44.261	61.336	1.00	20.15	8
	ATOM	1362	N	GLU A 197	23.649	43.519	59.454	1.00	18.90	7
25	ATOM	1363	OE2	GLU A 197	22.575	38.762	60.250	1.00	21.64	8
	ATOM	1364	OE1	GLU A 197	24.285	37.564	59.610	1.00	21.92	8
	ATOM	1365	CD	GLU A 197	23.784	38.629	59.811	1.00	20.50	6
	ATOM	1366	CG	GLU A 197	24.621	39.884	59.573	1.00	24.14	6
	ATOM	1367	CB	GLU A 197	23.810	41.138	59.266	1.00	19.21	6
30	ATOM	1368	CA	GLU A 197	24.642	42.460	59.266	1.00	22.15	6
	ATOM	1369	C	GLU A 197	25.599	42.545	58.109	1.00	18.74	6
	ATOM	1370	O	GLU A 197	26.761	42.130	58.148	1.00	17.44	8
	ATOM	1371	N	ILE A 198	25.090	43.096	56.996	1.00	17.50	7
	ATOM	1372	CD1	ILE A 198	28.230	41.260	54.456	1.00	17.58	6
35	ATOM	1373	CG1	ILE A 198	26.759	41.350	54.022	1.00	15.01	6
	ATOM	1374	CB	ILE A 198	25.746	41.660	55.141	1.00	17.39	6
	ATOM	1375	CG2	ILE A 198	24.381	41.337	54.553	1.00	14.58	6
	ATOM	1376	CA	ILE A 198	25.916	43.091	55.794	1.00	19.95	6
	ATOM	1377	C	ILE A 198	25.455	44.307	54.934	1.00	16.93	6
40	ATOM	1378	O	ILE A 198	24.294	44.655	55.167	1.00	18.25	8
	ATOM	1379	N	SER A 199	26.288	44.736	54.001	1.00	16.29	7
	ATOM	1380	OG	SER A 199	26.677	47.445	54.695	1.00	21.30	8
	ATOM	1381	CB	SER A 199	26.803	47.058	53.330	1.00	20.88	6
	ATOM	1382	CA	SER A 199	25.866	45.811	53.103	1.00	22.96	6
45	ATOM	1383	C	SER A 199	26.017	45.418	51.664	1.00	18.35	6
	ATOM	1384	O	SER A 199	26.885	44.606	51.311	1.00	17.27	8
	ATOM	1385	N	ALA A 200	25.292	46.082	50.773	1.00	17.99	7
	ATOM	1386	CB	ALA A 200	24.507	44.703	48.899	1.00	15.76	6
	ATOM	1387	CA	ALA A 200	25.488	45.800	49.315	1.00	14.75	6
50	ATOM	1388	C	ALA A 200	25.057	47.101	48.587	1.00	18.96	6
	ATOM	1389	O	ALA A 200	24.393	47.954	49.211	1.00	17.64	8
	ATOM	1390	N	PRO A 201	25.286	47.223	47.306	1.00	20.51	7
	ATOM	1391	CG	PRO A 201	26.661	47.136	45.380	1.00	18.80	6
	ATOM	1392	CD	PRO A 201	26.109	46.242	46.503	1.00	16.13	6
55	ATOM	1393	CB	PRO A 201	25.425	47.930	45.033	1.00	17.17	6
	ATOM	1394	CA	PRO A 201	24.903	48.309	46.424	1.00	17.87	6
	ATOM	1395	C	PRO A 201	23.380	48.465	46.492	1.00	19.20	6
	ATOM	1396	O	PRO A 201	22.635	47.530	46.248	1.00	18.47	8
	ATOM	1397	N	GLY A 202	22.926	49.697	46.814	1.00	17.75	7
60	ATOM	1398	CA	GLY A 202	21.523	49.979	46.902	1.00	17.51	6
	ATOM	1399	C	GLY A 202	21.097	51.274	46.221	1.00	14.82	6

358

	ATOM	1400	O	GLY	A	202	19.959	51.700	46.457	1.00	18.85	8
	ATOM	1401	N	VAL	A	203	21.915	51.907	45.439	1.00	16.17	7
	ATOM	1402	CG2	VAL	A	203	22.372	54.486	47.007	1.00	17.72	6
	ATOM	1403	CG1	VAL	A	203	22.264	55.632	44.833	1.00	25.37	6
5	ATOM	1404	CB	VAL	A	203	22.496	54.306	45.506	1.00	22.60	6
	ATOM	1405	CA	VAL	A	203	21.601	53.222	44.828	1.00	17.78	6
	ATOM	1406	C	VAL	A	203	21.846	53.134	43.307	1.00	16.42	6
	ATOM	1407	O	VAL	A	203	22.908	52.700	42.814	1.00	17.23	8
	ATOM	1408	N	ASN	A	204	20.759	53.554	42.619	1.00	16.98	7
10	ATOM	1409	ND2	ASN	A	204	22.049	56.884	41.402	1.00	24.32	7
	ATOM	1410	OD1	ASN	A	204	19.987	56.330	40.912	1.00	24.35	8
	ATOM	1411	CG	ASN	A	204	21.182	56.036	40.900	1.00	23.87	6
	ATOM	1412	CB	ASN	A	204	21.683	54.651	40.519	1.00	18.76	6
	ATOM	1413	CA	ASN	A	204	20.810	53.543	41.142	1.00	20.24	6
15	ATOM	1414	C	ASN	A	204	21.115	52.155	40.598	1.00	19.44	6
	ATOM	1415	O	ASN	A	204	22.059	52.014	39.793	1.00	19.05	8
	ATOM	1416	N	VAL	A	205	20.304	51.197	41.050	1.00	16.97	7
	ATOM	1417	CG2	VAL	A	205	21.243	49.080	42.914	1.00	19.48	6
	ATOM	1418	CG1	VAL	A	205	20.212	47.386	41.427	1.00	16.95	6
20	ATOM	1419	CB	VAL	A	205	20.268	48.874	41.764	1.00	19.38	6
	ATOM	1420	CA	VAL	A	205	20.599	49.801	40.597	1.00	16.59	6
	ATOM	1421	C	VAL	A	205	19.701	49.489	39.385	1.00	15.34	6
	ATOM	1422	O	VAL	A	205	18.461	49.433	39.519	1.00	16.76	8
	ATOM	1423	N	ASN	A	206	20.246	49.285	38.208	1.00	15.47	7
25	ATOM	1424	ND2	ASN	A	206	18.309	49.679	34.515	1.00	17.91	7
	ATOM	1425	OD1	ASN	A	206	20.019	48.800	33.506	1.00	25.78	8
	ATOM	1426	CG	ASN	A	206	19.539	49.313	34.549	1.00	20.79	6
	ATOM	1427	CB	ASN	A	206	20.396	49.386	35.803	1.00	18.19	6
	ATOM	1428	CA	ASN	A	206	19.494	48.977	37.009	1.00	15.81	6
30	ATOM	1429	C	ASN	A	206	19.179	47.474	37.041	1.00	18.98	6
	ATOM	1430	O	ASN	A	206	20.072	46.681	37.314	1.00	15.12	8
	ATOM	1431	N	SER	A	207	17.979	47.102	36.724	1.00	16.57	7
	ATOM	1432	OG	SER	A	207	17.057	43.866	37.998	1.00	15.36	8
	ATOM	1433	CB	SER	A	207	17.276	45.255	38.130	1.00	20.35	6
35	ATOM	1434	CA	SER	A	207	17.570	45.714	36.707	1.00	19.00	6
	ATOM	1435	C	SER	A	207	16.343	45.545	35.805	1.00	22.26	6
	ATOM	1436	O	SER	A	207	15.858	46.526	35.217	1.00	19.33	8
	ATOM	1437	N	THR	A	208	15.892	44.328	35.624	1.00	16.17	7
	ATOM	1438	CG2	THR	A	208	15.719	41.808	33.875	1.00	16.30	6
40	ATOM	1439	OG1	THR	A	208	14.725	41.939	36.073	1.00	18.37	8
	ATOM	1440	CB	THR	A	208	14.653	42.429	34.738	1.00	20.01	6
	ATOM	1441	CA	THR	A	208	14.750	43.997	34.777	1.00	18.19	6
	ATOM	1442	C	THR	A	208	13.490	44.637	35.310	1.00	16.06	6
	ATOM	1443	O	THR	A	208	13.445	44.830	36.515	1.00	18.68	8
45	ATOM	1444	N	TYR	A	209	12.439	44.866	34.532	1.00	15.66	7
	ATOM	1445	OH	TYR	A	209	8.061	49.995	38.353	1.00	24.32	8
	ATOM	1446	CD2	TYR	A	209	9.608	48.724	35.372	1.00	21.41	6
	ATOM	1447	CE2	TYR	A	209	8.725	49.455	36.172	1.00	18.95	6
	ATOM	1448	CZ	TYR	A	209	8.877	49.300	37.523	1.00	21.61	6
50	ATOM	1449	CE1	TYR	A	209	9.825	48.409	38.105	1.00	20.67	6
	ATOM	1450	CD1	TYR	A	209	10.663	47.694	37.280	1.00	16.67	6
	ATOM	1451	CG	TYR	A	209	10.576	47.859	35.859	1.00	20.84	6
	ATOM	1452	CB	TYR	A	209	11.535	47.084	34.944	1.00	14.85	6
	ATOM	1453	CA	TYR	A	209	11.262	45.514	35.051	1.00	17.74	6
55	ATOM	1454	C	TYR	A	209	10.037	45.095	34.241	1.00	18.77	6
	ATOM	1455	O	TYR	A	209	10.306	44.671	33.159	1.00	17.94	8
	ATOM	1456	N	THR	A	210	8.808	45.263	34.610	1.00	17.67	7
	ATOM	1457	CG2	THR	A	210	6.190	44.160	35.943	1.00	18.91	6
	ATOM	1458	OG1	THR	A	210	6.688	46.353	35.498	1.00	22.54	8
60	ATOM	1459	CB	THR	A	210	6.354	45.166	34.830	1.00	22.53	6
	ATOM	1460	CA	THR	A	210	7.576	44.936	33.961	1.00	15.65	6

359

	ATOM	1461	C	THR	A	210	7.530	45.630	32.615	1.00	21.08	6
	ATOM	1462	O	THR	A	210	8.245	46.596	32.337	1.00	21.96	8
	ATOM	1463	N	GLY	A	211	6.772	45.091	31.686	1.00	21.88	7
	ATOM	1464	CA	GLY	A	211	6.639	45.433	30.294	1.00	16.41	6
5	ATOM	1465	C	GLY	A	211	7.894	45.195	29.496	1.00	20.65	6
	ATOM	1466	O	GLY	A	211	8.073	45.931	28.520	1.00	21.25	8
	ATOM	1467	N	ASN	A	212	8.774	44.261	29.787	1.00	18.14	7
	ATOM	1468	ND2	ASN	A	212	10.850	42.997	25.498	1.00	19.71	7
	ATOM	1469	OD1	ASN	A	212	12.024	42.844	27.473	1.00	22.97	8
10	ATOM	1470	CG	ASN	A	212	10.949	43.075	26.839	1.00	24.04	6
	ATOM	1471	CB	ASN	A	212	9.727	43.459	27.633	1.00	20.82	6
	ATOM	1472	CA	ASN	A	212	9.992	44.039	29.021	1.00	18.32	6
	ATOM	1473	C	ASN	A	212	10.824	45.313	29.009	1.00	21.79	6
	ATOM	1474	O	ASN	A	212	11.338	45.759	27.979	1.00	19.34	8
15	ATOM	1475	N	ARG	A	213	11.135	45.871	30.138	1.00	22.53	7
	ATOM	1476	NH2	ARG	A	213	9.855	52.374	32.384	1.00	51.03	7
	ATOM	1477	NH1	ARG	A	213	7.807	51.315	33.080	1.00	51.09	7
	ATOM	1478	CZ	ARG	A	213	8.906	51.367	32.264	1.00	58.97	6
	ATOM	1479	NE	ARG	A	213	9.162	50.491	31.270	1.00	46.66	7
20	ATOM	1480	CD	ARG	A	213	8.664	49.317	30.665	1.00	34.39	6
	ATOM	1481	CG	ARG	A	213	9.943	48.779	30.110	1.00	26.79	6
	ATOM	1482	CB	ARG	A	213	11.019	48.187	30.997	1.00	18.34	6
	ATOM	1483	CA	ARG	A	213	11.923	47.139	30.309	1.00	21.06	6
	ATOM	1484	C	ARG	A	213	13.028	46.814	31.301	1.00	20.29	6
25	ATOM	1485	O	ARG	A	213	13.179	45.698	31.856	1.00	22.59	8
	ATOM	1486	N	TYR	A	214	13.880	47.783	31.513	1.00	19.32	7
	ATOM	1487	OH	TYR	A	214	16.343	42.867	28.955	1.00	22.20	8
	ATOM	1488	CD2	TYR	A	214	16.835	45.207	31.687	1.00	21.30	6
	ATOM	1489	CE2	TYR	A	214	16.827	44.005	30.925	1.00	25.13	6
30	ATOM	1490	CZ	TYR	A	214	16.378	44.008	29.622	1.00	22.68	6
	ATOM	1491	CE1	TYR	A	214	15.989	45.194	29.017	1.00	22.65	6
	ATOM	1492	CD1	TYR	A	214	15.994	46.390	29.760	1.00	27.11	6
	ATOM	1493	CG	TYR	A	214	16.429	46.385	31.106	1.00	20.60	6
	ATOM	1494	CB	TYR	A	214	16.401	47.666	31.882	1.00	20.69	6
35	ATOM	1495	CA	TYR	A	214	15.005	47.781	32.462	1.00	19.05	6
	ATOM	1496	C	TYR	A	214	14.859	49.127	33.171	1.00	27.90	6
	ATOM	1497	O	TYR	A	214	14.650	50.072	32.408	1.00	24.71	8
	ATOM	1498	N	VAL	A	215	14.933	49.316	34.454	1.00	20.15	7
	ATOM	1499	CG2	VAL	A	215	13.057	52.183	35.930	1.00	35.02	6
40	ATOM	1500	CG1	VAL	A	215	12.963	50.184	36.901	1.00	21.12	6
	ATOM	1501	CB	VAL	A	215	13.309	50.726	35.561	1.00	22.47	6
	ATOM	1502	CA	VAL	A	215	14.790	50.566	35.197	1.00	19.28	6
	ATOM	1503	C	VAL	A	215	15.780	50.613	36.352	1.00	26.25	6
	ATOM	1504	O	VAL	A	215	16.115	49.538	36.921	1.00	18.11	8
45	ATOM	1505	N	SER	A	216	16.242	51.836	36.638	1.00	18.21	7
	ATOM	1506	OG	SER	A	216	18.922	53.199	38.291	1.00	28.38	8
	ATOM	1507	CB	SER	A	216	18.437	52.619	37.132	1.00	19.54	6
	ATOM	1508	CA	SER	A	216	17.173	52.022	37.788	1.00	14.76	6
	ATOM	1509	C	SER	A	216	16.379	52.452	38.994	1.00	15.93	6
50	ATOM	1510	O	SER	A	216	15.417	53.260	38.998	1.00	17.76	8
	ATOM	1511	N	LEU	A	217	16.536	51.851	40.157	1.00	15.32	7
	ATOM	1512	CD2	LEU	A	217	12.758	52.145	40.632	1.00	14.71	6
	ATOM	1513	CD1	LEU	A	217	12.750	49.735	41.258	1.00	16.65	6
	ATOM	1514	CG	LEU	A	217	13.614	50.916	40.808	1.00	16.11	6
55	ATOM	1515	CB	LEU	A	217	14.725	51.092	41.795	1.00	14.31	6
	ATOM	1516	CA	LEU	A	217	15.935	51.919	41.450	1.00	15.26	6
	ATOM	1517	C	LEU	A	217	16.939	51.939	42.603	1.00	15.62	6
	ATOM	1518	O	LEU	A	217	18.064	51.549	42.450	1.00	16.30	8
	ATOM	1519	N	SER	A	218	16.586	52.646	43.680	1.00	20.18	7
60	ATOM	1520	OG	SER	A	218	18.487	54.649	44.162	1.00	18.87	8
	ATOM	1521	CB	SER	A	218	17.616	54.260	45.170	1.00	14.18	6

360

	ATOM	1522	CA	SER	A	218	17.407	52.767	44.891	1.00	15.36	6
	ATOM	1523	C	SER	A	218	16.603	52.333	46.074	1.00	11.18	6
	ATOM	1524	O	SER	A	218	15.384	52.612	46.252	1.00	15.32	8
	ATOM	1525	N	GLY	A	219	17.294	51.647	46.946	1.00	13.73	7
5	ATOM	1526	CA	GLY	A	219	16.541	51.213	48.130	1.00	14.10	6
	ATOM	1527	C	GLY	A	219	17.263	50.056	48.790	1.00	13.62	6
	ATOM	1528	O	GLY	A	219	18.107	49.446	48.142	1.00	15.45	8
	ATOM	1529	N	THR	A	220	16.951	49.763	50.039	1.00	17.44	7
	ATOM	1530	CG2	THR	A	220	18.258	49.511	52.936	1.00	16.33	6
10	ATOM	1531	OG1	THR	A	220	15.916	48.713	52.516	1.00	15.80	8
	ATOM	1532	CB	THR	A	220	17.286	48.535	52.245	1.00	14.42	6
	ATOM	1533	CA	THR	A	220	17.461	48.580	50.735	1.00	17.50	6
	ATOM	1534	C	THR	A	220	16.870	47.306	50.022	1.00	19.97	6
	ATOM	1535	O	THR	A	220	17.485	46.256	50.007	1.00	15.81	8
15	ATOM	1536	N	SER	A	221	15.767	47.403	49.310	1.00	20.24	7
	ATOM	1537	OG	SER	A	221	12.959	47.113	48.642	1.00	16.51	8
	ATOM	1538	CB	SER	A	221	13.930	46.838	47.667	1.00	14.33	6
	ATOM	1539	CA	SER	A	221	15.123	46.390	48.506	1.00	12.90	6
	ATOM	1540	C	SER	A	221	16.074	46.003	47.362	1.00	15.12	6
20	ATOM	1541	O	SER	A	221	15.824	44.880	46.946	1.00	17.53	8
	ATOM	1542	N	MET	A	222	16.866	46.875	46.831	1.00	17.58	7
	ATOM	1543	CE	MET	A	222	14.201	47.976	44.365	1.00	20.40	6
	ATOM	1544	SD	MET	A	222	15.531	48.968	44.957	1.00	19.45	16
	ATOM	1545	CG	MET	A	222	17.005	48.396	44.101	1.00	13.71	6
25	ATOM	1546	CB	MET	A	222	18.168	47.953	44.968	1.00	15.78	6
	ATOM	1547	CA	MET	A	222	17.828	46.631	45.753	1.00	16.66	6
	ATOM	1548	C	MET	A	222	19.114	46.047	46.344	1.00	18.62	6
	ATOM	1549	O	MET	A	222	19.914	45.476	45.641	1.00	18.19	8
	ATOM	1550	N	ALA	A	223	19.567	46.403	47.559	1.00	18.17	7
30	ATOM	1551	CB	ALA	A	223	21.100	46.725	49.390	1.00	15.52	6
	ATOM	1552	CA	ALA	A	223	20.798	45.907	48.119	1.00	17.73	6
	ATOM	1553	C	ALA	A	223	20.550	44.390	48.476	1.00	18.17	6
	ATOM	1554	O	ALA	A	223	21.442	43.582	48.237	1.00	15.32	8
	ATOM	1555	N	THR	A	224	19.505	43.993	49.096	1.00	15.05	7
35	ATOM	1556	CG2	THR	A	224	17.181	41.475	50.592	1.00	17.28	6
	ATOM	1557	OG1	THR	A	224	17.567	43.643	51.132	1.00	18.23	8
	ATOM	1558	CB	THR	A	224	17.580	42.815	49.991	1.00	19.73	6
	ATOM	1559	CA	THR	A	224	19.045	42.695	49.562	1.00	16.32	6
	ATOM	1560	C	THR	A	224	19.361	41.608	48.529	1.00	18.10	6
40	ATOM	1561	O	THR	A	224	20.139	40.681	48.831	1.00	18.64	8
	ATOM	1562	N	PRO	A	225	18.887	41.707	47.295	1.00	19.05	7
	ATOM	1563	CG	PRO	A	225	18.136	42.513	45.242	1.00	14.92	6
	ATOM	1564	CD	PRO	A	225	17.891	42.669	46.729	1.00	13.28	6
	ATOM	1565	CB	PRO	A	225	18.243	40.998	45.078	1.00	16.78	6
45	ATOM	1566	CA	PRO	A	225	19.095	40.659	46.305	1.00	16.58	6
	ATOM	1567	C	PRO	A	225	20.555	40.511	46.005	1.00	18.27	6
	ATOM	1568	O	PRO	A	225	20.931	39.450	45.449	1.00	18.34	8
	ATOM	1569	N	HIS	A	226	21.430	41.465	46.154	1.00	14.72	7
	ATOM	1570	CD2	HIS	A	226	24.294	43.788	43.752	1.00	18.07	6
50	ATOM	1571	NE2	HIS	A	226	23.748	44.863	43.075	1.00	19.52	7
	ATOM	1572	CE1	HIS	A	226	22.668	45.150	43.774	1.00	14.51	6
	ATOM	1573	ND1	HIS	A	226	22.493	44.451	44.863	1.00	17.68	7
	ATOM	1574	CG	HIS	A	226	23.536	43.522	44.843	1.00	17.79	6
	ATOM	1575	CB	HIS	A	226	23.792	42.501	45.921	1.00	16.33	6
55	ATOM	1576	CA	HIS	A	226	22.850	41.289	45.803	1.00	15.23	6
	ATOM	1577	C	HIS	A	226	23.338	40.212	46.774	1.00	16.97	6
	ATOM	1578	O	HIS	A	226	24.229	39.428	46.452	1.00	18.46	8
	ATOM	1579	N	VAL	A	227	22.891	40.288	48.000	1.00	16.78	7
	ATOM	1580	CG2	VAL	A	227	23.890	41.135	50.680	1.00	14.40	6
60	ATOM	1581	CG1	VAL	A	227	23.403	38.803	51.556	1.00	16.55	6
	ATOM	1582	CB	VAL	A	227	23.078	39.851	50.480	1.00	16.86	6

361

	ATOM	1583	CA	VAL	A	227	23.317	39.328	49.058	1.00	19.40	6
	ATOM	1584	C	VAL	A	227	22.622	37.966	48.813	1.00	18.81	6
	ATOM	1585	O	VAL	A	227	23.389	37.026	48.945	1.00	18.80	8
	ATOM	1586	N	ALA	A	228	21.341	37.929	48.499	1.00	16.53	7
5	ATOM	1587	CB	ALA	A	228	19.234	36.911	47.825	1.00	14.14	6
	ATOM	1588	CA	ALA	A	228	20.698	36.697	48.134	1.00	15.92	6
	ATOM	1589	C	ALA	A	228	21.468	36.063	46.986	1.00	18.89	6
	ATOM	1590	O	ALA	A	228	21.717	34.844	46.986	1.00	18.57	8
	ATOM	1591	N	GLY	A	229	21.867	36.825	45.976	1.00	17.16	7
10	ATOM	1592	CA	GLY	A	229	22.612	36.385	44.821	1.00	18.29	6
	ATOM	1593	C	GLY	A	229	23.921	35.754	45.298	1.00	20.93	6
	ATOM	1594	O	GLY	A	229	24.368	34.727	44.804	1.00	18.60	8
	ATOM	1595	N	VAL	A	230	24.721	36.337	46.178	1.00	19.63	7
	ATOM	1596	CG2	VAL	A	230	27.344	37.967	46.499	1.00	16.69	6
15	ATOM	1597	CG1	VAL	A	230	28.071	36.209	48.063	1.00	17.82	6
	ATOM	1598	CB	VAL	A	230	26.831	36.870	47.428	1.00	18.91	6
	ATOM	1599	CA	VAL	A	230	25.995	35.834	46.650	1.00	21.31	6
	ATOM	1600	C	VAL	A	230	25.729	34.506	47.398	1.00	20.36	6
	ATOM	1601	O	VAL	A	230	26.608	33.630	47.327	1.00	18.14	8
20	ATOM	1602	N	ALA	A	231	24.704	34.423	48.186	1.00	16.19	7
	ATOM	1603	CB	ALA	A	231	23.099	33.453	49.852	1.00	15.64	6
	ATOM	1604	CA	ALA	A	231	24.303	33.272	48.943	1.00	19.23	6
	ATOM	1605	C	ALA	A	231	24.106	32.150	47.878	1.00	26.28	6
	ATOM	1606	O	ALA	A	231	24.646	31.063	48.051	1.00	18.81	8
25	ATOM	1607	N	ALA	A	232	23.425	32.341	46.769	1.00	23.31	7
	ATOM	1608	CB	ALA	A	232	22.170	31.917	44.677	1.00	17.17	6
	ATOM	1609	CA	ALA	A	232	23.190	31.406	45.678	1.00	19.51	6
	ATOM	1610	C	ALA	A	232	24.513	30.938	45.055	1.00	22.03	6
	ATOM	1611	O	ALA	A	232	24.669	29.709	44.797	1.00	21.60	8
30	ATOM	1612	N	LEU	A	233	25.450	31.831	44.890	1.00	18.00	7
	ATOM	1613	CD2	LEU	A	233	27.058	32.978	41.722	1.00	19.56	6
	ATOM	1614	CD1	LEU	A	233	28.229	34.741	42.822	1.00	22.23	6
	ATOM	1615	CG	LEU	A	233	27.261	33.626	43.063	1.00	25.68	6
	ATOM	1616	CB	LEU	A	233	27.734	32.638	44.100	1.00	19.00	6
35	ATOM	1617	CA	LEU	A	233	26.758	31.512	44.380	1.00	19.18	6
	ATOM	1618	C	LEU	A	233	27.478	30.583	45.399	1.00	32.14	6
	ATOM	1619	O	LEU	A	233	28.163	29.617	44.985	1.00	26.65	8
	ATOM	1620	N	VAL	A	234	27.417	30.811	46.694	1.00	23.95	7
	ATOM	1621	CG2	VAL	A	234	28.911	31.915	49.153	1.00	20.13	6
40	ATOM	1622	CG1	VAL	A	234	28.484	29.847	50.295	1.00	18.16	6
	ATOM	1623	CB	VAL	A	234	28.054	30.627	49.104	1.00	20.46	6
	ATOM	1624	CA	VAL	A	234	28.187	30.033	47.683	1.00	20.36	6
	ATOM	1625	C	VAL	A	234	27.586	28.631	47.676	1.00	21.66	6
	ATOM	1626	O	VAL	A	234	28.344	27.700	47.665	1.00	22.83	8
45	ATOM	1627	N	LYS	A	235	26.274	28.546	47.694	1.00	21.98	7
	ATOM	1628	NZ	LYS	A	235	22.620	23.743	50.078	1.00	29.40	7
	ATOM	1629	CE	LYS	A	235	22.462	24.483	48.842	1.00	25.24	6
	ATOM	1630	CD	LYS	A	235	23.510	25.553	48.797	1.00	30.16	6
	ATOM	1631	CG	LYS	A	235	23.079	26.379	47.585	1.00	26.63	6
50	ATOM	1632	CB	LYS	A	235	23.988	27.625	47.594	1.00	22.69	6
	ATOM	1633	CA	LYS	A	235	25.469	27.337	47.688	1.00	27.46	6
	ATOM	1634	C	LYS	A	235	25.907	26.501	46.462	1.00	34.52	6
	ATOM	1635	O	LYS	A	235	26.029	25.292	46.590	1.00	26.32	8
	ATOM	1636	N	SER	A	236	26.101	27.082	45.314	1.00	23.34	7
55	ATOM	1637	OG	SER	A	236	27.255	28.235	42.597	1.00	24.48	8
	ATOM	1638	CB	SER	A	236	26.224	27.305	42.840	1.00	20.87	6
	ATOM	1639	CA	SER	A	236	26.457	26.441	44.069	1.00	27.78	6
	ATOM	1640	C	SER	A	236	27.893	25.932	44.239	1.00	32.81	6
	ATOM	1641	O	SER	A	236	28.289	24.881	43.697	1.00	33.79	8
60	ATOM	1642	N	ARG	A	237	28.779	26.633	44.889	1.00	27.94	7
	ATOM	1643	NH2	ARG	A	237	36.693	26.015	46.199	1.00	43.63	7

362

	ATOM	1644	NH1	ARG	A	237	34.671	24.734	46.068	1.00	50.62	7
	ATOM	1645	CZ	ARG	A	237	35.394	25.866	45.921	1.00	55.63	6
	ATOM	1646	NE	ARG	A	237	34.768	26.943	45.423	1.00	45.03	7
	ATOM	1647	CD	ARG	A	237	33.356	26.880	44.981	1.00	35.54	6
5	ATOM	1648	CG	ARG	A	237	32.431	27.220	46.107	1.00	36.78	6
	ATOM	1649	CB	ARG	A	237	31.048	27.417	45.451	1.00	36.35	6
	ATOM	1650	CA	ARG	A	237	30.183	26.229	45.057	1.00	30.62	6
	ATOM	1651	C	ARG	A	237	30.294	25.177	46.187	1.00	37.26	6
	ATOM	1652	O	ARG	A	237	31.226	24.364	46.081	1.00	32.10	8
10	ATOM	1653	N	TYR	A	238	29.478	25.193	47.202	1.00	25.70	7
	ATOM	1654	OH	TYR	A	238	35.377	26.896	48.995	1.00	38.64	8
	ATOM	1655	CD2	TYR	A	238	31.736	26.903	49.223	1.00	26.69	6
	ATOM	1656	CE2	TYR	A	238	33.029	27.369	49.095	1.00	30.27	6
	ATOM	1657	CZ	TYR	A	238	34.086	26.481	49.141	1.00	38.00	6
15	ATOM	1658	CE1	TYR	A	238	33.828	25.135	49.328	1.00	33.49	6
	ATOM	1659	CD1	TYR	A	238	32.531	24.676	49.487	1.00	30.85	6
	ATOM	1660	CG	TYR	A	238	31.457	25.546	49.441	1.00	33.19	6
	ATOM	1661	CB	TYR	A	238	30.081	24.961	49.606	1.00	24.64	6
	ATOM	1662	CA	TYR	A	238	29.529	24.325	48.331	1.00	23.05	6
20	ATOM	1663	C	TYR	A	238	28.122	23.867	48.656	1.00	25.25	6
	ATOM	1664	O	TYR	A	238	27.514	24.266	49.659	1.00	30.61	8
	ATOM	1665	N	PRO	A	239	27.688	22.920	47.848	1.00	27.20	7
	ATOM	1666	CG	PRO	A	239	27.396	21.618	45.894	1.00	27.10	6
	ATOM	1667	CD	PRO	A	239	28.420	22.386	46.677	1.00	28.97	6
25	ATOM	1668	CB	PRO	A	239	26.237	21.401	46.789	1.00	27.07	6
	ATOM	1669	CA	PRO	A	239	26.374	22.336	47.936	1.00	24.32	6
	ATOM	1670	C	PRO	A	239	26.018	21.775	49.271	1.00	27.11	6
	ATOM	1671	O	PRO	A	239	24.832	21.805	49.646	1.00	34.83	8
	ATOM	1672	N	SER	A	240	27.032	21.338	49.983	1.00	28.71	7
30	ATOM	1673	OG	SER	A	240	28.905	20.696	51.933	1.00	44.71	8
	ATOM	1674	CB	SER	A	240	27.802	19.807	51.651	1.00	32.98	6
	ATOM	1675	CA	SER	A	240	26.658	20.772	51.295	1.00	31.35	6
	ATOM	1676	C	SER	A	240	26.514	21.852	52.339	1.00	35.23	6
	ATOM	1677	O	SER	A	240	26.021	21.373	53.361	1.00	33.72	8
35	ATOM	1678	N	TYR	A	241	26.917	23.099	52.126	1.00	33.49	7
	ATOM	1679	OH	TYR	A	241	32.514	26.940	53.424	1.00	39.30	8
	ATOM	1680	CD2	TYR	A	241	28.974	26.952	52.686	1.00	28.15	6
	ATOM	1681	CE2	TYR	A	241	30.301	27.321	52.920	1.00	31.52	6
	ATOM	1682	CZ	TYR	A	241	31.258	26.429	53.256	1.00	27.23	6
40	ATOM	1683	CE1	TYR	A	241	30.883	25.121	53.346	1.00	31.25	6
	ATOM	1684	CD1	TYR	A	241	29.567	24.730	53.129	1.00	37.65	6
	ATOM	1685	CG	TYR	A	241	28.574	25.641	52.769	1.00	33.89	6
	ATOM	1686	CB	TYR	A	241	27.141	25.321	52.486	1.00	29.46	6
	ATOM	1687	CA	TYR	A	241	26.737	24.060	53.228	1.00	26.63	6
45	ATOM	1688	C	TYR	A	241	25.346	24.320	53.736	1.00	25.38	6
	ATOM	1689	O	TYR	A	241	24.430	24.339	52.874	1.00	29.17	8
	ATOM	1690	N	THR	A	242	25.146	24.489	55.044	1.00	24.62	7
	ATOM	1691	CG2	THR	A	242	23.731	22.950	57.120	1.00	42.00	6
	ATOM	1692	OG1	THR	A	242	24.567	25.108	57.591	1.00	31.68	8
50	ATOM	1693	CB	THR	A	242	23.519	24.442	56.951	1.00	33.79	6
	ATOM	1694	CA	THR	A	242	23.802	24.846	55.488	1.00	26.63	6
	ATOM	1695	C	THR	A	242	23.625	26.399	55.366	1.00	31.00	6
	ATOM	1696	O	THR	A	242	24.567	27.112	55.026	1.00	24.59	8
	ATOM	1697	N	ASN	A	243	22.455	26.868	55.672	1.00	23.08	7
55	ATOM	1698	ND2	ASN	A	243	19.284	28.150	58.303	1.00	25.05	7
	ATOM	1699	OD1	ASN	A	243	21.147	26.869	58.079	1.00	29.80	8
	ATOM	1700	CG	ASN	A	243	20.365	27.718	57.665	1.00	31.10	6
	ATOM	1701	CB	ASN	A	243	20.705	28.307	56.289	1.00	26.51	6
	ATOM	1702	CA	ASN	A	243	22.106	28.250	55.754	1.00	21.33	6
60	ATOM	1703	C	ASN	A	243	23.148	28.899	56.695	1.00	27.65	6
	ATOM	1704	O	ASN	A	243	23.802	29.886	56.362	1.00	25.83	8

363

	ATOM	1705	N	ASN	A	244	23.468	28.330	57.867	1.00	26.03	7
	ATOM	1706	ND2	ASN	A	244	22.587	29.649	60.811	1.00	34.79	7
	ATOM	1707	OD1	ASN	A	244	22.802	27.357	61.068	1.00	44.84	8
	ATOM	1708	CG	ASN	A	244	23.191	28.490	60.756	1.00	35.31	6
5	ATOM	1709	CB	ASN	A	244	24.543	28.310	60.131	1.00	23.50	6
	ATOM	1710	CA	ASN	A	244	24.468	28.913	58.741	1.00	23.28	6
	ATOM	1711	C	ASN	A	244	25.852	29.042	58.177	1.00	20.60	6
	ATOM	1712	O	ASN	A	244	26.588	29.986	58.528	1.00	25.11	8
	ATOM	1713	N	GLN	A	245	26.288	28.065	57.405	1.00	26.52	7
10	ATOM	1714	NE2	GLN	A	245	29.731	23.917	56.910	1.00	46.58	7
	ATOM	1715	OE1	GLN	A	245	27.592	23.848	56.288	1.00	31.92	8
	ATOM	1716	CD	GLN	A	245	28.495	24.413	56.857	1.00	31.91	6
	ATOM	1717	CG	GLN	A	245	28.158	25.769	57.424	1.00	31.13	6
	ATOM	1718	CB	GLN	A	245	28.079	26.789	56.257	1.00	22.63	6
15	ATOM	1719	CA	GLN	A	245	27.641	28.159	56.822	1.00	25.01	6
	ATOM	1720	C	GLN	A	245	27.700	29.217	55.724	1.00	22.64	6
	ATOM	1721	O	GLN	A	245	28.812	29.747	55.628	1.00	22.33	8
	ATOM	1722	N	ILE	A	246	26.579	29.386	55.029	1.00	19.17	7
	ATOM	1723	CD1	ILE	A	246	24.121	28.540	51.765	1.00	24.28	6
20	ATOM	1724	CG1	ILE	A	246	25.491	28.913	52.305	1.00	22.92	6
	ATOM	1725	CB	ILE	A	246	25.388	30.250	53.066	1.00	24.08	6
	ATOM	1726	CG2	ILE	A	246	25.359	31.365	52.019	1.00	15.42	6
	ATOM	1727	CA	ILE	A	246	26.626	30.376	53.946	1.00	20.73	6
	ATOM	1728	C	ILE	A	246	26.625	31.770	54.595	1.00	21.00	6
25	ATOM	1729	O	ILE	A	246	27.450	32.600	54.231	1.00	21.98	8
	ATOM	1730	N	ARG	A	247	25.815	31.946	55.595	1.00	17.95	7
	ATOM	1731	NH2	ARG	A	247	21.172	36.496	61.002	1.00	23.24	7
	ATOM	1732	NH1	ARG	A	247	20.813	34.285	60.509	1.00	25.64	7
	ATOM	1733	CZ	ARG	A	247	21.541	35.380	60.384	1.00	22.42	6
30	ATOM	1734	NE	ARG	A	247	22.621	35.221	59.659	1.00	20.46	7
	ATOM	1735	CD	ARG	A	247	23.075	33.985	59.041	1.00	23.20	6
	ATOM	1736	CG	ARG	A	247	24.278	34.245	58.197	1.00	24.44	6
	ATOM	1737	CB	ARG	A	247	24.599	32.992	57.408	1.00	19.57	6
	ATOM	1738	CA	ARG	A	247	25.664	33.174	56.359	1.00	19.19	6
35	ATOM	1739	C	ARG	A	247	27.002	33.597	56.927	1.00	25.09	6
	ATOM	1740	O	ARG	A	247	27.519	34.707	56.756	1.00	22.02	8
	ATOM	1741	N	GLN	A	248	27.650	32.632	57.527	1.00	20.56	7
	ATOM	1742	NE2	GLN	A	248	31.226	29.317	59.418	1.00	42.74	7
	ATOM	1743	OE1	GLN	A	248	30.871	30.465	61.389	1.00	46.93	8
40	ATOM	1744	CD	GLN	A	248	30.990	30.383	60.165	1.00	51.60	6
	ATOM	1745	CG	GLN	A	248	30.736	31.700	59.458	1.00	35.08	6
	ATOM	1746	CB	GLN	A	248	29.288	31.684	59.012	1.00	25.69	6
	ATOM	1747	CA	GLN	A	248	28.981	32.908	58.114	1.00	22.60	6
	ATOM	1748	C	GLN	A	248	30.017	33.161	57.069	1.00	21.32	6
45	ATOM	1749	O	GLN	A	248	30.901	33.970	57.349	1.00	21.82	8
	ATOM	1750	N	ARG	A	249	29.967	32.465	55.934	1.00	19.01	7
	ATOM	1751	NH2	ARG	A	249	35.824	29.779	51.206	1.00	40.53	7
	ATOM	1752	NH1	ARG	A	249	34.826	29.545	53.357	1.00	37.14	7
	ATOM	1753	CZ	ARG	A	249	34.844	29.899	52.078	1.00	40.51	6
50	ATOM	1754	NE	ARG	A	249	33.791	30.659	51.779	1.00	39.30	7
	ATOM	1755	CD	ARG	A	249	33.221	31.268	52.999	1.00	30.94	6
	ATOM	1756	CG	ARG	A	249	31.842	31.741	52.706	1.00	25.20	6
	ATOM	1757	CB	ARG	A	249	30.911	31.639	53.899	1.00	22.51	6
	ATOM	1758	CA	ARG	A	249	31.014	32.723	54.960	1.00	22.86	6
55	ATOM	1759	C	ARG	A	249	30.910	34.140	54.373	1.00	17.79	6
	ATOM	1760	O	ARG	A	249	31.962	34.746	54.144	1.00	19.55	8
	ATOM	1761	N	ILE	A	250	29.677	34.545	54.142	1.00	18.51	7
	ATOM	1762	CD1	ILE	A	250	26.140	35.220	51.913	1.00	18.42	6
	ATOM	1763	CG1	ILE	A	250	27.639	35.352	52.018	1.00	18.91	6
60	ATOM	1764	CB	ILE	A	250	27.933	36.102	53.316	1.00	23.88	6
	ATOM	1765	CG2	ILE	A	250	27.571	37.586	53.219	1.00	20.31	6

364

	ATOM	1766	CA	ILE	A	250	29.420	35.892	53.596	1.00	22.42	6
	ATOM	1767	C	ILE	A	250	29.936	36.914	54.662	1.00	20.70	6
	ATOM	1768	O	ILE	A	250	30.697	37.770	54.268	1.00	20.41	8
	ATOM	1769	N	ASN	A	251	29.611	36.778	55.909	1.00	16.56	7
5	ATOM	1770	ND2	ASN	A	251	27.085	37.213	59.132	1.00	21.27	7
	ATOM	1771	OD1	ASN	A	251	27.518	38.556	57.396	1.00	20.03	8
	ATOM	1772	CG	ASN	A	251	27.884	37.722	58.234	1.00	20.12	6
	ATOM	1773	CB	ASN	A	251	29.340	37.365	58.291	1.00	14.58	6
	ATOM	1774	CA	ASN	A	251	30.053	37.650	56.988	1.00	19.60	6
10	ATOM	1775	C	ASN	A	251	31.548	37.703	57.148	1.00	22.53	6
	ATOM	1776	O	ASN	A	251	32.201	38.759	57.273	1.00	20.64	8
	ATOM	1777	N	GLN	A	252	32.182	36.536	57.064	1.00	22.96	7
	ATOM	1778	NE2	GLN	A	252	33.954	32.143	57.495	1.00	26.44	7
	ATOM	1779	OE1	GLN	A	252	34.257	32.144	59.601	1.00	34.65	8
15	ATOM	1780	CD	GLN	A	252	33.983	32.794	58.626	1.00	33.52	6
	ATOM	1781	CG	GLN	A	252	33.666	34.300	58.676	1.00	32.35	6
	ATOM	1782	CB	GLN	A	252	34.161	35.012	57.438	1.00	19.97	6
	ATOM	1783	CA	GLN	A	252	33.609	36.444	57.294	1.00	22.53	6
	ATOM	1784	C	GLN	A	252	34.428	37.094	56.208	1.00	21.65	6
20	ATOM	1785	O	GLN	A	252	35.605	37.391	56.464	1.00	22.97	8
	ATOM	1786	N	THR	A	253	33.896	37.119	55.011	1.00	18.20	7
	ATOM	1787	CG2	THR	A	253	35.122	35.288	53.155	1.00	26.77	6
	ATOM	1788	OG1	THR	A	253	33.244	36.457	52.335	1.00	20.36	8
	ATOM	1789	CB	THR	A	253	34.578	36.633	52.726	1.00	22.10	6
25	ATOM	1790	CA	THR	A	253	34.689	37.647	53.898	1.00	19.73	6
	ATOM	1791	C	THR	A	253	34.340	39.071	53.463	1.00	19.95	6
	ATOM	1792	O	THR	A	253	34.913	39.503	52.482	1.00	20.25	8
	ATOM	1793	N	ALA	A	254	33.429	39.726	54.117	1.00	19.64	7
	ATOM	1794	CB	ALA	A	254	31.740	41.369	54.617	1.00	18.36	6
30	ATOM	1795	CA	ALA	A	254	32.987	41.091	53.782	1.00	24.84	6
	ATOM	1796	C	ALA	A	254	34.120	42.087	53.921	1.00	21.63	6
	ATOM	1797	O	ALA	A	254	35.058	41.906	54.708	1.00	20.05	8
	ATOM	1798	N	THR	A	255	34.176	43.140	53.147	1.00	21.26	7
	ATOM	1799	CG2	THR	A	255	36.230	46.013	52.142	1.00	25.89	6
35	ATOM	1800	OG1	THR	A	255	35.698	44.059	51.035	1.00	26.17	8
	ATOM	1801	CB	THR	A	255	35.139	44.994	51.925	1.00	22.57	6
	ATOM	1802	CA	THR	A	255	35.193	44.192	53.240	1.00	21.57	6
	ATOM	1803	C	THR	A	255	34.718	45.197	54.248	1.00	19.15	6
	ATOM	1804	O	THR	A	255	33.550	45.592	54.161	1.00	19.14	8
40	ATOM	1805	N	TYR	A	256	35.458	45.555	55.262	1.00	21.54	7
	ATOM	1806	OH	TYR	A	256	35.344	50.333	61.399	1.00	27.67	8
	ATOM	1807	CD2	TYR	A	256	35.133	47.291	59.487	1.00	18.22	6
	ATOM	1808	CE2	TYR	A	256	34.941	48.186	60.527	1.00	19.33	6
	ATOM	1809	CZ	TYR	A	256	35.581	49.413	60.435	1.00	23.39	6
45	ATOM	1810	CE1	TYR	A	256	36.360	49.758	59.359	1.00	21.50	6
	ATOM	1811	CD1	TYR	A	256	36.542	48.786	58.359	1.00	24.73	6
	ATOM	1812	CG	TYR	A	256	35.930	47.528	58.414	1.00	18.54	6
	ATOM	1813	CB	TYR	A	256	36.204	46.511	57.365	1.00	19.73	6
	ATOM	1814	CA	TYR	A	256	35.044	46.469	56.350	1.00	22.62	6
50	ATOM	1815	C	TYR	A	256	34.821	47.867	55.744	1.00	21.73	6
	ATOM	1816	O	TYR	A	256	35.663	48.297	54.920	1.00	21.87	8
	ATOM	1817	N	LEU	A	257	33.684	48.448	56.082	1.00	19.62	7
	ATOM	1818	CD2	LEU	A	257	32.720	49.475	52.464	1.00	18.99	6
	ATOM	1819	CD1	LEU	A	257	30.367	48.966	53.151	1.00	21.76	6
55	ATOM	1820	CG	LEU	A	257	31.817	48.960	53.516	1.00	20.48	6
	ATOM	1821	CB	LEU	A	257	31.922	49.666	54.836	1.00	19.04	6
	ATOM	1822	CA	LEU	A	257	33.313	49.753	55.519	1.00	27.37	6
	ATOM	1823	C	LEU	A	257	33.263	50.866	56.576	1.00	27.50	6
	ATOM	1824	O	LEU	A	257	33.107	52.015	56.207	1.00	25.78	8
60	ATOM	1825	N	GLY	A	258	33.152	50.534	57.828	1.00	22.89	7
	ATOM	1826	CA	GLY	A	258	33.057	51.513	58.894	1.00	21.99	6

365

	ATOM	1827	C	GLY A 258	32.163	50.880	59.937	1.00	24.86	6
	ATOM	1828	O	GLY A 258	31.926	49.672	60.084	1.00	24.18	8
	ATOM	1829	N	SER A 259	31.569	51.743	60.724	1.00	20.88	7
	ATOM	1830	OG	SER A 259	29.158	52.213	63.426	1.00	31.61	8
5	ATOM	1831	CB	SER A 259	29.974	52.583	62.307	1.00	24.36	6
	ATOM	1832	CA	SER A 259	30.733	51.337	61.822	1.00	24.45	6
	ATOM	1833	C	SER A 259	29.770	50.171	61.540	1.00	27.60	6
	ATOM	1834	O	SER A 259	28.843	50.318	60.730	1.00	22.13	8
	ATOM	1835	N	PRO A 260	29.842	49.141	62.343	1.00	21.74	7
10	ATOM	1836	CG	PRO A 260	31.036	47.393	63.408	1.00	24.82	6
	ATOM	1837	CD	PRO A 260	30.994	48.911	63.310	1.00	25.73	6
	ATOM	1838	CB	PRO A 260	29.514	47.117	63.404	1.00	21.61	6
	ATOM	1839	CA	PRO A 260	29.031	47.947	62.217	1.00	19.23	6
	ATOM	1840	C	PRO A 260	27.609	48.328	62.386	1.00	21.40	6
15	ATOM	1841	O	PRO A 260	26.757	47.607	61.855	1.00	21.68	8
	ATOM	1842	N	SER A 261	27.313	49.416	63.117	1.00	24.57	7
	ATOM	1843	OG	SER A 261	26.184	51.724	64.185	1.00	39.92	8
	ATOM	1844	CB	SER A 261	25.584	50.471	64.588	1.00	26.32	6
	ATOM	1845	CA	SER A 261	25.846	49.736	63.266	1.00	21.73	6
20	ATOM	1846	C	SER A 261	25.265	50.281	61.945	1.00	22.12	6
	ATOM	1847	O	SER A 261	24.035	50.276	61.642	1.00	24.01	8
	ATOM	1848	N	LEU A 262	26.160	50.717	61.066	1.00	16.86	7
	ATOM	1849	CD2	LEU A 262	25.190	53.985	60.792	1.00	19.12	6
	ATOM	1850	CD1	LEU A 262	27.301	54.691	59.591	1.00	24.54	6
25	ATOM	1851	CG	LEU A 262	26.558	53.641	60.336	1.00	21.01	6
	ATOM	1852	CB	LEU A 262	26.462	52.472	59.338	1.00	17.76	6
	ATOM	1853	CA	LEU A 262	25.690	51.214	59.777	1.00	20.61	6
	ATOM	1854	C	LEU A 262	25.743	50.137	58.665	1.00	22.18	6
	ATOM	1855	O	LEU A 262	24.898	50.044	57.784	1.00	20.95	8
30	ATOM	1856	N	TYR A 263	26.839	49.424	58.640	1.00	20.86	7
	ATOM	1857	OH	TYR A 263	29.102	54.204	55.461	1.00	26.47	8
	ATOM	1858	CD2	TYR A 263	29.566	51.211	57.467	1.00	26.53	6
	ATOM	1859	CE2	TYR A 263	29.687	52.535	57.088	1.00	20.10	6
	ATOM	1860	CZ	TYR A 263	28.983	52.914	55.953	1.00	29.82	6
35	ATOM	1861	CE1	TYR A 263	28.242	51.962	55.229	1.00	23.04	6
	ATOM	1862	CD1	TYR A 263	28.099	50.658	55.660	1.00	21.97	6
	ATOM	1863	CG	TYR A 263	28.770	50.273	56.804	1.00	22.44	6
	ATOM	1864	CB	TYR A 263	28.675	48.901	57.334	1.00	18.72	6
	ATOM	1865	CA	TYR A 263	27.257	48.431	57.689	1.00	19.34	6
40	ATOM	1866	C	TYR A 263	27.356	46.941	58.112	1.00	20.55	6
	ATOM	1867	O	TYR A 263	27.557	46.151	57.208	1.00	20.61	8
	ATOM	1868	N	GLY A 264	27.252	46.559	59.371	1.00	23.63	7
	ATOM	1869	CA	GLY A 264	27.399	45.182	59.846	1.00	23.19	6
	ATOM	1870	C	GLY A 264	28.879	44.821	59.611	1.00	20.89	6
45	ATOM	1871	O	GLY A 264	29.792	45.612	59.912	1.00	22.16	8
	ATOM	1872	N	ASN A 265	29.016	43.657	58.986	1.00	20.54	7
	ATOM	1873	ND2	ASN A 265	28.705	40.460	59.762	1.00	18.28	7
	ATOM	1874	OD1	ASN A 265	31.001	40.510	60.158	1.00	22.08	8
	ATOM	1875	CG	ASN A 265	29.953	40.799	59.474	1.00	23.12	6
50	ATOM	1876	CB	ASN A 265	30.177	41.671	58.249	1.00	22.83	6
	ATOM	1877	CA	ASN A 265	30.354	43.162	58.629	1.00	18.37	6
	ATOM	1878	C	ASN A 265	30.933	43.918	57.463	1.00	17.82	6
	ATOM	1879	O	ASN A 265	32.101	43.734	57.184	1.00	19.89	8
	ATOM	1880	N	GLY A 266	30.149	44.653	56.673	1.00	18.61	7
55	ATOM	1881	CA	GLY A 266	30.810	45.365	55.570	1.00	16.52	6
	ATOM	1882	C	GLY A 266	30.147	44.955	54.258	1.00	14.39	6
	ATOM	1883	O	GLY A 266	29.012	44.489	54.261	1.00	17.41	8
	ATOM	1884	N	LEU A 267	30.938	45.180	53.248	1.00	17.00	7
	ATOM	1885	CD2	LEU A 267	31.818	46.464	48.528	1.00	20.10	6
60	ATOM	1886	CD1	LEU A 267	29.447	46.337	49.267	1.00	17.92	6
	ATOM	1887	CG	LEU A 267	30.836	45.825	49.468	1.00	21.20	6

	ATOM	1888	CB	LEU	A	267	31.195	45.897	50.957	1.00	17.31	6
	ATOM	1889	CA	LEU	A	267	30.473	44.933	51.911	1.00	19.77	6
	ATOM	1890	C	LEU	A	267	30.613	43.483	51.457	1.00	19.17	6
	ATOM	1891	O	LEU	A	267	31.713	43.027	51.499	1.00	18.24	8
5	ATOM	1892	N	VAL	A	268	29.515	42.890	51.007	1.00	18.79	7
	ATOM	1893	CG2	VAL	A	268	28.108	39.597	49.871	1.00	21.18	6
	ATOM	1894	CG1	VAL	A	268	27.562	41.922	48.977	1.00	17.72	6
	ATOM	1895	CB	VAL	A	268	28.154	41.080	50.102	1.00	18.91	6
	ATOM	1896	CA	VAL	A	268	29.593	41.487	50.497	1.00	19.33	6
10	ATOM	1897	C	VAL	A	268	30.656	41.429	49.439	1.00	21.97	6
	ATOM	1898	O	VAL	A	268	30.848	42.376	48.631	1.00	22.07	8
	ATOM	1899	N	HIS	A	269	31.459	40.358	49.345	1.00	18.59	7
	ATOM	1900	CD2	HIS	A	269	36.030	41.419	48.127	1.00	21.57	6
	ATOM	1901	NE2	HIS	A	269	36.783	41.004	47.080	1.00	22.77	7
15	ATOM	1902	CE1	HIS	A	269	36.264	39.941	46.468	1.00	21.29	6
	ATOM	1903	ND1	HIS	A	269	35.180	39.655	47.082	1.00	20.84	7
	ATOM	1904	CG	HIS	A	269	35.025	40.514	48.132	1.00	18.43	6
	ATOM	1905	CB	HIS	A	269	33.878	40.370	49.071	1.00	17.66	6
	ATOM	1906	CA	HIS	A	269	32.544	40.254	48.361	1.00	19.72	6
20	ATOM	1907	C	HIS	A	269	32.331	38.894	47.726	1.00	22.36	6
	ATOM	1908	O	HIS	A	269	32.629	37.899	48.397	1.00	20.96	8
	ATOM	1909	N	ALA	A	270	31.766	38.818	46.559	1.00	22.28	7
	ATOM	1910	CB	ALA	A	270	30.573	37.918	44.601	1.00	17.20	6
	ATOM	1911	CA	ALA	A	270	31.431	37.593	45.842	1.00	21.32	6
25	ATOM	1912	C	ALA	A	270	32.677	36.745	45.532	1.00	26.89	6
	ATOM	1913	O	ALA	A	270	32.564	35.516	45.514	1.00	23.48	8
	ATOM	1914	N	GLY	A	271	33.851	37.281	45.257	1.00	20.68	7
	ATOM	1915	CA	GLY	A	271	35.107	36.638	44.880	1.00	24.34	6
	ATOM	1916	C	GLY	A	271	35.612	35.980	46.150	1.00	30.38	6
30	ATOM	1917	O	GLY	A	271	35.866	34.786	46.145	1.00	29.87	8
	ATOM	1918	N	ARG	A	272	35.718	36.672	47.271	1.00	25.63	7
	ATOM	1919	NH2	ARG	A	272	39.216	41.988	51.543	1.00	39.62	7
	ATOM	1920	NH1	ARG	A	272	37.245	41.084	52.031	1.00	33.73	7
	ATOM	1921	CZ	ARG	A	272	38.322	41.035	51.261	1.00	29.01	6
35	ATOM	1922	NE	ARG	A	272	38.462	40.006	50.408	1.00	27.85	7
	ATOM	1923	CD	ARG	A	272	37.427	38.979	50.545	1.00	24.30	6
	ATOM	1924	CG	ARG	A	272	37.529	37.929	49.449	1.00	24.96	6
	ATOM	1925	CB	ARG	A	272	36.387	36.959	49.653	1.00	24.60	6
	ATOM	1926	CA	ARG	A	272	36.154	35.998	48.480	1.00	24.91	6
40	ATOM	1927	C	ARG	A	272	35.202	34.911	48.922	1.00	26.40	6
	ATOM	1928	O	ARG	A	272	35.641	33.851	49.431	1.00	28.24	8
	ATOM	1929	N	ALA	A	273	33.914	35.188	48.929	1.00	19.69	7
	ATOM	1930	CB	ALA	A	273	31.517	34.902	49.536	1.00	20.87	6
	ATOM	1931	CA	ALA	A	273	32.936	34.244	49.474	1.00	24.30	6
45	ATOM	1932	C	ALA	A	273	32.968	32.852	48.766	1.00	27.05	6
	ATOM	1933	O	ALA	A	273	32.536	31.854	49.362	1.00	24.22	8
	ATOM	1934	N	THR	A	274	33.319	32.767	47.501	1.00	24.53	7
	ATOM	1935	CG2	THR	A	274	31.085	32.479	45.548	1.00	21.97	6
	ATOM	1936	OG1	THR	A	274	33.334	32.912	44.673	1.00	21.52	8
50	ATOM	1937	CB	THR	A	274	32.493	32.003	45.307	1.00	23.92	6
	ATOM	1938	CA	THR	A	274	33.266	31.637	46.614	1.00	27.06	6
	ATOM	1939	C	THR	A	274	34.616	30.968	46.450	1.00	23.60	6
	ATOM	1940	O	THR	A	274	34.742	30.024	45.712	1.00	26.35	8
	ATOM	1941	N	GLN	A	275	35.613	31.466	47.075	1.00	24.38	7
55	ATOM	1942	NE2	GLN	A	275	38.108	33.169	50.922	1.00	25.89	7
	ATOM	1943	OE1	GLN	A	275	39.935	31.618	50.540	1.00	44.50	8
	ATOM	1944	CD	GLN	A	275	38.904	32.283	50.229	1.00	56.87	6
	ATOM	1945	CG	GLN	A	275	38.801	31.879	48.740	1.00	54.09	6
	ATOM	1946	CB	GLN	A	275	37.513	31.251	48.481	1.00	27.55	6
60	ATOM	1947	CA	GLN	A	275	36.966	30.920	47.124	1.00	31.54	6
	ATOM	1948	C	GLN	A	275	36.688	29.412	47.422	1.00	38.56	6

367

ATOM	1949	O	GLN A 275	37.587	28.549	47.205	1.00	37.01	8
ATOM	1950	OE	GLN A 275	36.105	29.125	48.479	1.00	31.65	8

CLAIMS

1. A method of selecting a protein variant having modified immunogenicity as compared to a parent protein, comprising the
5 steps of:

- a) obtaining antibody binding peptide sequences,
- b) using the sequences to localise epitope sequences on the 3-
10 dimensional structure of the parent protein,
- c) defining an epitope area including amino acids situated within 5 Å from the epitope amino acids constituting the epitope sequence,
15
- d) changing one or more of the amino acids defining the epitope area of the parent protein by genetic engineering mutations of a DNA sequence encoding the parent protein,
- 20 e) introducing the mutated DNA sequence into a suitable host, culturing said host and expressing the protein variant, and
- f) evaluating the immunogenicity of the protein variant using
25 the parent protein as reference.

2. The method according to claim 1, wherein the sequences of step a) are obtained by screening a random peptide display package library with antibodies raised against any protein of interest and sequencing the amino acid sequence of the antibody binding peptide, or the DNA sequence encoding the antibody binding peptide.
30

3. The method according to claim 2, wherein antibodies for screening the random peptide display package library are raised against the parent protein.
- 5 4. The method according to claims 2-3, wherein the peptide display package library is a phage display library.
5. The method according to claims 2-4, wherein the peptides of the peptide display package library are oligopeptides having
10 from 5 to 25 amino acids.
6. The method according to claim 1, wherein the antibody binding peptide sequences of step a) are obtained by screening a library of known peptides related to the primary sequence of any protein
15 of interest, with antibodies raised against the protein of interest.
7. The method according to any of the preceding claims, wherein epitope patterns are identified by sequence alignment of anti-
20 body binding peptide sequences and these epitope patterns are used to guide localisation of epitope sequences on the 3-dimensional structure of the parent protein.
8. The method according to any of the preceding claims, wherein
25 the epitope area of step c) equals the epitope sequence.
9. The method according to any of the preceding claims, wherein hot spot amino acids of the parent protein are identified.
- 30 10. The method according to any of the preceding claims, wherein the epitope area is changed by substituting, adding and/or deleting at least one amino acid of the epitope area.

11. The method according to claim 10, wherein the epitope area is changed by substituting, adding and/or deleting at least one hot spot amino acid.
- 5 12. The method according to claims 10-11, wherein amino acids in the epitope area are changed by substituting and/or inserting at least one amino acid by an amino acid which render the substituted and/or inserted amino acid a target for covalent conjugation to an activated polymer.
- 10 13. the method according to claim 12, wherein the amino acid for substitution and/or insertion is selected from the group consisting of K, C, D, E.
- 15 14. The method according to claim 12, wherein the molecule for covalent conjugation is selected from the group of activated synthetic or natural polymers.
15. The method according to claim 14, wherein the activated syn-
20 thetic polymer is a polyethylene glycol.
16. The method according to any of the preceding claims, wherein the immunogenicity is measured by competitive ELISA.
- 25 17. The method according to any of the preseding claims, wherein the protein variant has reduced allergenicity.
18. The method according to claim 17, wherein the allergenicity of the protein variant is below 75%, preferably below 50%, more
30 preferably below 25% of the allergenicity of the parent protein.
19. The method according to any of the preceding claims, wherein the parent protein is an enzyme or an environmental allergen or a pharmaceutical protein.

20. The method according to claim 19, wherein the enzyme is selected from the group consisting of glycosyl hydrolases, carbohydrases, peroxidases, proteases, lipolytic enzymes, phytases, polysaccharide lyases, oxidoreductases, transglutaminases and glucoseisomerases.

21. The method according to claim 19, wherein the environmental allergen is selected from the group consisting of pollen, dust mites, mammals, venoms, fungi, food allergens or other plant allergens.

22. A protein variant obtainable by a method according to claims 1-21.

15

23. A protein variant, wherein the amino acid sequence of the protein variant differs from the amino acid sequence of the parent protein with respect to at least one epitope area of the parent protein.

20

24. The protein variant according to claim 23 having modified immunogenicity as compared to its parent protein.

25. A protein variant according to claims 22-24, wherein the epitope areas are defined on the parent protein structure by being localised less than 5 Å from any of the following epitope patterns: P > S/T D P G; P > > D A G; > P > R D T G; P > S/T D P G; > R Y > K/R; > R S A; > G > > A G; V H > G >; A > I D P R/K; A R > A; Q > Y > D >; > P > > A P > S; R/K R F > N; D/E Q I F F T; A > > > > Y P >; L > G R S; R P P R; > E Y; > P > > P A P > S; > K L > >; K Q S; > K L > >; Y I > K L; R Q > > D/E; N > > E L.

26. The protein variant according to claims 22 or 23, wherein the epitope areas correspond to antibody binding peptide sequences reactive to antibodies raised against the parent protein.

5

27. The protein variant according to claims 22-26, wherein the epitope pattern is a IgE epitope pattern.

28. The protein variant according to claims 22-27, wherein at
10 least one hot spot amino acid is substituted or deleted.

29. The protein variant according to claims 22-28, wherein the allergenicity of the protein variant is below 75%, preferable below 50%, more preferably below 25% of the allergenicity of the
15 parent protein.

30. The protein variant according to claims 22-29, wherein the protein variant is an environmental allergen, preferable an allergen selected from the group consisting of pollen, dust mites,
20 mammals, venoms, fungi, food allergens or other plant allergens.

31. The protein variant according to claims 22-29, wherein the protein variant is an antifungal peptide or antimicrobial peptide.

25

32. The protein variant according to claim 30, wherein the allergen is pollen allergen comprising one or more of the following substitutions corresponding to any of the following in SEQ ID NO 6:

30

Position	T	10	to	A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W, Y;
Position	V	12	to	A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, Y;

373

Position P 14 to A, C, D, E, F, G, H, I, K, L, M,
 N, Q, R, S, T, V, W, Y;
 Position A 16 to C, D, E, F, G, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 5 Position R 17 to A, C, D, E, F, G, H, I, K, L, M,
 N, P, Q, S, T, V, W, Y;
 Position K 20 to A, C, D, E, F, G, H, I, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position L 24 to A, C, D, E, F, G, H, I, K, M, N,
 10 P, Q, R, S, T, V, W, Y;
 Position F 30 to A, C, D, E, G, H, I, K, L, M, N,
 P, Q, R, S, T, W, Y;
 Position P 31 to A, C, D, E, F, G, H, I, K, L, M,
 N, Q, R, S, T, V, W, Y;
 15 Position K 32 to A, C, D, E, F, G, H, I, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position A 34 to C, D, E, F, G, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position P 35 to A, C, D, E, F, G, H, I, K, L, M,
 20 N, Q, R, S, T, V, W, Y;
 Position Q 36 to A, C, D, E, F, G, H, I, K, L, M,
 N, P, R, S, T, V, W, Y;
 Position A 37 to C, D, E, F, G, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 25 Position S 39 to A, C, D, E, F, G, H, I, K, L, M,
 N, P, Q, R, T, V, W, Y;
 Position S 40 to A, C, D, E, F, G, H, I, K, L, M,
 N, P, Q, R, T, V, W, Y;
 Position E 42 to A, C, D, F, G, H, I, K, L, M, N,
 30 P, Q, R, S, T, V, W, Y;
 Position S 57 to A, C, D, E, F, G, H, I, K, L, M,
 P, Q, R, T, V, W, Y;
 Position F 58 to A, C, D, E, G, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;

374

Position P 59 to A, C, D, E, F, G, H, I, K, L, M,
N, Q, R, S, T, V, W, Y;

Position E 60 to A, C, D, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

5 Position G 61 to A, C, D, E, F, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position L 62 to A, C, D, E, F, G, H, I, K, M, N,
P, Q, R, S, T, V, W, Y;

Position P 63 to A, C, D, E, F, G, H, I, K, L, M,
10 N, Q, R, S, T, V, W, Y;

Position F 64 to A, C, D, E, G, H, I, K, L, M,
N, P, Q, R, S, T, V, W, Y;

Position K 65 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

15 Position T 77 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, V, W, Y;

Position F 79 to A, C, D, E, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position P 90 to A, C, D, E, F, G, H, I, K, L, M,
20 N, Q, R, S, T, V, W, Y;

Position D 93 to A, C, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position V 105 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, W, Y;

25 Position A 106 to C, D, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position T 107 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, V, W, Y;

Position D 109 to A, C, E, F, G, H, I, K, L, M, N,
30 P, Q, R, S, T, V, W, Y;

Position G 110 to A, C, D, E, F, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position K 123 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

375

Position E 127 to A, C, D, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position K 129 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

5 Position E 131 to A, C, D, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position S 136 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, T, V, W, Y;

Position G 140 to A, C, D, E, F, H, I, K, L, M, N,
10 P, Q, R, S, T, V, W, Y;

Position L 143 to A, C, D, E, F, G, H, I, K, M, N,
P, Q, R, S, T, V, W, Y;

Position R 145 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, S, T, V, W, Y;

15 Position S 149 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, T, V, W, Y;

Position Y 150 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, V, W;

Position L 152 to A, C, D, E, F, G, H, I, K, M, N,
20 P, Q, R, S, T, V, W, Y;

Position A 153 to C, D, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position D 156 to A, C, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

25 Position Y 158 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, V, W;

33. The protein variant according to claim 32, wherein the pol-
len allergen comprises one or more of the following substitu-
30 tions:

position P31 to A, G, L, or S;

position A34 to D, E, F, H, K, N, P, Q, R, W, or Y;

position P35 to A, G, L, or S;

376

position A37 to D, E, F, H, K, N, P, Q, R, W, or Y;
 position S39 to D, E, F, H, K, N, P, Q, R, W, or Y;
 position S40 to D, E, F, H, K, N, P, Q, R, W, or Y;
 position P59 to A, G, L, or S;
 5 position L62 to D, E, F, H, K, N, P, Q, R, W, or Y;
 position P63 to A, G, L, or S.

34. The allergen according to claims 32-33, wherein the allergen has at least 81%, preferably at least 85%, more preferably at least 90%, even more preferably at least 96%, most preferably at least 98% homology to SEQ ID NO 6.

35. The pollen allergen according to claim 34, wherein the allergen has the amino acid sequence of SEQ ID NO 6.

15

36. The protein variant according to claim 30, wherein the allergen is mite allergen comprising one or more of the following substitutions corresponding to any of the following in SEQ ID NO 7:

20

Position D 1 to A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
 Position Q 2 to A, C, D, E, F, G, H, I, K, L, M, N, P, R, S, T, V, W, Y;
 25 Position N 11 to A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, Y;
 Position E 12 to A, C, D, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
 Position K 14 to A, C, D, E, F, G, H, I, L, M, N, P, Q, R, S, T, V, W, Y;
 30 Position K 15 to A, C, D, E, F, G, H, I, L, M, N, P, Q, R, S, T, V, W, Y;
 Position D 19 to A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;

377

Position G 20 to A, C, D, E, F, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position H 30 to A, C, D, E, F, G, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

5 Position R 31 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, S, T, V, W, Y;

Position G 32 to A, C, D, E, F, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position P 34 to A, C, D, E, F, G, H, I, K, L, M,
10 N, Q, R, S, T, V, W, Y;

Position T 36 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, V, W, Y;

Position L 37 to A, C, D, E, F, G, H, I, K, M,
N, P, Q, R, S, T, V, W, Y;

15 Position E 38 to A, C, D, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position A 39 to C, D, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position L 40 to A, C, D, E, F, G, H, I, K, M, N,
20 P, Q, R, S, T, V, W, Y;

Position D 59 to A, C, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position L 61 to A, C, D, E, F, G, H, I, K, M, N,
P, Q, R, S, T, V, W, Y;

25 Position E 62 to A, C, D, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position D 64 to A, C, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position N 71 to A, C, D, E, F, G, H, I, K, L, M,
30 P, Q, R, S, T, V, W, Y;

Position H 74 to A, C, D, E, F, G, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position F 75 to A, C, D, E, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

378

Position P 79 to A, C, D, E, F, G, H, I, K, L, M,
N, Q, R, S, T, V, W, Y;

Position Q 85 to A, C, D, E, F, G, H, I, K, L, M,
N, P, R, S, T, V, W, Y;

5 Position D 87 to A, C, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position Y 90 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, V, W;

Position T 91 to A, C, D, E, F, G, H, I, K, L, M,
10 N, P, Q, R, S, V, W, Y;

Position W 92 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, V, Y;

Position N 93 to A, C, D, E, F, G, H, I, K, L, M,
P, Q, R, S, T, V, W, Y;

15 Position P 95 to A, C, D, E, F, G, H, I, K, L, M,
N, Q, R, S, T, V, W, Y;

Position K 96 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

Position I 97 to A, C, D, E, F, G, H, K, L, M, N,
20 P, Q, R, S, T, V, W, Y;

Position A 98 to C, D, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position P 99 to A, C, D, E, F, G, H, I, K, L, M,
N, Q, R, S, T, V, W, Y;

25 Position S 101 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, T, V, W, Y;

Position E 102 to A, C, D, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position T 123 to A, C, D, E, F, G, H, I, K, L, M,
30 N, P, Q, R, S, V, W, Y;

Position K 126 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

Position R 128 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, S, T, V, W, Y;

379

Position D 129 to A, C, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

37. The mite allergen according to claims 36, wherein the aller-
5 gen has at least 81%, preferably at least 85%, more preferably
at least 90%, even more preferably at least 96%, most preferably
at least 98% homology to SEQ ID NO 7.

38. The mite allergen according to claim 37, wherein the aller-
10 gen has the amino acid sequence of SEQ ID NO 7.

39. The protein variant according to claim 30, wherein the al-
lergen is mite allergen comprising one or more of the following
substitutions corresponding to any of the following in SEQ ID NO
15 8:

Position L 17 to A, C, D, E, F, G, H, I, K, M, N,
P, Q, R, S, T, V, W, Y;

Position P 19 to A, C, D, E, F, G, H, I, K, L, M,
20 N, Q, R, S, T, V, W, Y;

Position G 20 to A, C, D, E, F, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position P 26 to A, C, D, E, F, G, H, I, K, L, M,
N, Q, R, S, T, V, W, Y;

25 Position I 28 to A, C, D, E, F, G, H, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position H 30 to A, C, D, E, F, G, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position R 31 to A, C, D, E, F, G, H, I, K, L, M,
30 N, P, Q, S, T, V, W, Y;

Position P 34 to A, C, D, E, F, G, H, I, K, L, M,
N, Q, R, S, T, V, W, Y;

Position F 35 to A, C, D, E, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position Q 36 to A, C, D, E, F, G, H, I, K, L, M,
 N, P, R, S, T, V, W, Y;
 Position K 55 to A, C, D, E, F, G, H, I, L, M, N,
 P, Q, R, S, T, V, W, Y;
 5 Position A 56 to C, D, E, F, G, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position S 57 to A, C, D, E, F, G, H, I, K, L, M,
 N, P, Q, R, T, V, W, Y;
 Position D 59 to A, C, E, F, G, H, I, K, L, M, N,
 10 P, Q, R, S, T, V, W, Y;
 Position G 60 to A, C, D, E, F, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position L 61 to A, C, D, E, F, G, H, I, K, M, N,
 P, Q, R, S, T, V, W, Y;
 15 Position E 62 to A, C, D, F, G, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position D 64 to A, C, E, F, G, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position P 66 to A, C, D, E, F, G, H, I, K, L, M,
 20 N, Q, R, S, T, V, W, Y;
 Position D 69 to A, C, E, F, G, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position K 89 to A, C, D, E, F, G, H, I, L, M, N,
 P, Q, R, S, T, V, W, Y;
 25 Position Y 90 to A, C, D, E, F, G, H, I, K, L, M,
 N, P, Q, R, S, T, V, W;
 Position T 91 to A, C, D, E, F, G, H, I, K, L, M,
 N, P, Q, R, S, V, W, Y;
 Position W 92 to A, C, D, E, F, G, H, I, K, L, M,
 30 N, P, Q, R, S, T, V, Y;
 Position P 95 to A, C, D, E, F, G, H, I, K, L, M,
 N, Q, R, S, T, V, W, Y;
 Position K 96 to A, C, D, E, F, G, H, I, L, M, N,
 P, Q, R, S, T, V, W, Y;

381

Position I 97 to A, C, D, E, F, G, H, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position P 99 to A, C, D, E, F, G, H, I, K, L, M,
N, Q, R, S, T, V, W, Y;

5 Position K 100 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

Position E 102 to A, C, D, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position N 103 to A, C, D, E, F, G, H, I, K, L, M,
10 P, Q, R, S, T, V, W, Y;

Position T 123 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, V, W, Y;

Position A 125 to C, D, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

15 Position R 128 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, S, T, V, W, Y;

40. The mite allergen according to claims 39, wherein the aller-
gen has at least 81%, preferably at least 85%, more preferably
20 at least 90%, even more preferably at least 96%, most preferably
at least 98% homology to SEQ ID NO 8.

41. The mite allergen according to claim 40, wherein the aller-
gen has the amino acid sequence of SEQ ID NO 8.

25

42. The protein variant according to claim 30, wherein the al-
lergen comprises one or more of the following substitutions cor-
responding to any of the following in SEQ ID NO 9:

Position V 1 to A, C, D, E, F, G, H, I, K, L, M,
30 N, P, Q, R, S, T, W, Y;

Position E 9 to A, C, D, F, G, H, I, K, L, M,
N, P, Q, R, S, T, V, W, Y;

Position K 10 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

382

Position N 13 to A, C, D, E, F, G, H, I, K, L, M,
P, Q, R, S, T, V, W, Y;

Position E 14 to A, C, D, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

5 Position K 15 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

Position H 16 to A, C, D, E, F, G, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position A 18 to C, D, E, F, G, H, I, K, L, M, N,
10 P, Q, R, S, T, V, W, Y;

Position R 34 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, S, T, V, W, Y;

Position H 36 to A, C, D, E, F, G, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

15 Position G 37 to A, C, D, E, F, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position S 38 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, T, V, W, Y;

Position W 41 to A, C, D, E, F, G, H, I, K, L, M,
20 N, P, Q, R, S, T, V, Y;

Position V 42 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, W, Y;

Position A 43 to C, D, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

25 Position F 54 to A, C, D, E, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position D 55 to A, C, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position S 56 to A, C, D, E, F, G, H, I, K, L, M,
30 N, P, Q, R, T, V, W, Y;

Position E 57 to A, C, D, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position P 59 to A, C, D, E, F, G, H, I, K, L, M,
N, Q, R, S, T, V, W, Y;

383

Position L 60 to A, C, D, E, F, G, H, I, K, M, N,
P, Q, R, S, T, V, W, Y;

Position Q 61 to A, C, D, E, F, G, H, I, K, L, M,
N, P, R, S, T, V, W, Y;

5 Position P 63 to A, C, D, E, F, G, H, I, K, L, M,
N, Q, R, S, T, V, W, Y;

Position R 67 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, S, T, V, W, Y;

Position L 69 to A, C, D, E, F, G, H, I, K, M, N,
10 P, Q, R, S, T, V, W, Y;

Position D 79 to A, C, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position E 84 to A, C, D, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

15 Position K 85 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

Position T 87 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, V, W, Y;

Position P 94 to A, C, D, E, F, G, H, I, K, L, M,
20 N, Q, R, S, T, V, W, Y;

43. The allergen according to claims 42, wherein the allergen
has at least 81%, preferably at least 85%, more preferably at
least 90%, even more preferably at least 96%, most preferably at
25 least 98% homology to SEQ ID NO 9.

44. The allergen according to claim 43, wherein the allergen has
the amino acid sequence of SEQ ID NO 9.

45. The protein variant according to claim 30, wherein the al-
30 lergen comprises one or more of the following substitutions cor-
responding to any of the following in SEQ ID NO 12:

Position I 1 to A, C, D, E, F, G, H, K, L, M, N,
P, Q, R, S, T, V, W, Y;

384

Position D 18 to A, C, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position D 41 to A, C, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

5 Position E 43 to A, C, D, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position K 65 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

Position Y 70 to A, C, D, E, F, G, H, I, K, L, M,
10 N, P, Q, R, S, T, V, W;

Position K 113 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

Position G 114 to A, C, D, E, F, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

15 Position S 116 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, T, V, W, Y;

Position P 119 to A, C, D, E, F, G, H, I, K, L, M,
N, Q, R, S, T, V, W, Y;

Position E 120 to A, C, D, F, G, H, I, K, L, M, N,
20 P, Q, R, S, T, V, W, Y;

Position L 122 to A, C, D, E, F, G, H, I, K, M, N,
P, Q, R, S, T, V, W, Y;

Position K 124 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

25 Position Q 126 to A, C, D, E, F, G, H, I, K, L, M,
N, P, R, S, T, V, W, Y;

Position Q 127 to A, C, D, E, F, G, H, I, K, L, M,
N, P, R, S, T, V, W, Y;

Position S 130 to A, C, D, E, F, G, H, I, K, L, M,
30 N, P, Q, R, T, V, W, Y;

Position R 132 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, S, T, V, W, Y;

Position I 139 to A, C, D, E, F, G, H, K, L, M, N,
P, Q, R, S, T, V, W, Y;

385

Position I 143 to A, C, D, E, F, G, H, K, L, M, N,
P, Q, R, S, T, V, W, Y;

46. The allergen according to claims 45, wherein the allergen
5 has at least 81%, preferably at least 85%, more preferably at
least 90%, even more preferably at least 96%, most preferably at
least 98% homology to SEQ ID NO 12.

47. The allergen according to claim 46, wherein the allergen has
10 the amino acid sequence of SEQ ID NO 12.

48. The protein variant according to claim 30, wherein the al-
lergen is a mammal allergen comprising one or more of the fol-
lowing substitutions corresponding to any of the following in
15 SEQ ID NO 13:

Position S 9 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, T, V, W, Y;

Position S 12 to A, C, D, E, F, G, H, I, K, L, M,
20 N, P, Q, R, T, V, W, Y;

Position Y 16 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, V, W;

Position D 23 to A, C, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

25 Position V 40 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, W, Y;

Position R 42 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, S, T, V, W, Y;

Position A 43 to C, D, E, F, G, H, I, K, L, M, N,
30 P, Q, R, S, T, V, W, Y;

Position L 44 to A, C, D, E, F, G, H, I, K, M,
N, P, Q, R, S, T, V, W, Y;

Position Y 50 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, V, W;

386

Position D 69 to A, C, E, F, G, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position N 80 to A, C, D, E, F, G, H, I, K, L, M,
 P, Q, R, S, T, V, W, Y;
 5 Position Y 84 to A, C, D, E, F, G, H, I, K, L, M,
 N, P, Q, R, S, T, V, W;
 Position P 110 to A, C, D, E, F, G, H, I, K, L, M,
 N, Q, R, S, T, V, W, Y;
 Position Q 112 to A, C, D, E, F, G, H, I, K, L, M,
 10 N, P, R, S, T, V, W, Y;
 Position E 120 to A, C, D, F, G, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position P 121 to A, C, D, E, F, G, H, I, K, L, M,
 N, Q, R, S, T, V, W, Y;
 15 Position D 122 to A, C, E, F, G, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position E 129 to A, C, D, F, G, H, I, K, L, M, N,
 P, Q, R, T, V, W, Y;
 Position K 133 to A, C, D, E, F, G, H, I, L, M, N,
 20 P, Q, R, S, T, V, W, Y;
 Position G 139 to A, C, D, E, F, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position K 142 to A, C, D, E, F, G, H, I, L, M, N,
 P, Q, R, S, T, V, W, Y;
 25 Position Q 156 to A, C, D, E, F, G, H, I, K, L, M,
 N, P, R, S, T, V, W, Y;
 Position L 157 to A, C, D, E, F, G, H, I, K, M, N,
 P, Q, R, S, T, V, W, Y;
 Position R 158 to A, C, D, E, F, G, H, I, K, L, M,
 30 N, P, Q, S, T, V, W, Y;
 Position G 159 to A, C, D, E, F, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;

49. The allergen according to claims 48, wherein the allergen has at least 81%, preferably at least 85%, more preferably at least 90%, even more preferably at least 96%, most preferably at least 98% homology to SEQ ID NO 13.

5

50. The allergen according to claim 49, wherein the allergen has the amino acid sequence of SEQ ID NO 13.

51. The protein variant according to claim 30, wherein the allergen comprises one or more of the following substitutions corresponding to any of the following in SEQ ID NO 15:

	Position	K	1	to	A, C, D, E, F, G, H, I, L, M, N, P, Q, R, S, T, V, W, Y;
15	Position	S	24	to	A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W, Y;
	Position	E	35	to	A, C, D, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
20	Position	R	45	to	A, C, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, Y;
	Position	T	47	to	A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W, Y;
	Position	D	52	to	A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
25	Position	Y	53	to	A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W;
	Position	N	59	to	A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, Y;
30	Position	R	61	to	A, C, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, Y;
	Position	W	62	to	A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, Y;
	Position	W	63	to	A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, Y;

Position N 65 to A, C, D, E, F, G, H, I, K, L, M,
P, Q, R, S, T, V, W, Y;

Position D 66 to A, C, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

5 Position G 67 to A, C, D, E, F, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position P 70 to A, C, D, E, F, G, H, I, K, L, M,
N, Q, R, S, T, V, W, Y;

Position S 72 to A, C, D, E, F, G, H, I, K, L, M,
10 N, P, Q, R, T, V, W, Y;

Position R 73 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, S, T, V, W, Y;

Position L 75 to A, C, D, E, F, G, H, I, K, M, N,
P, Q, R, S, T, V, W, Y;

15 Position I 78 to A, C, D, E, F, G, H, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position P 79 to A, C, D, E, F, G, H, I, K, L, M,
N, Q, R, S, T, V, W, Y;

Position S 81 to A, C, D, E, F, G, H, I, K, L, M,
20 N, P, Q, R, T, V, W, Y;

Position A 82 to C, D, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position L 84 to A, C, D, E, F, G, H, I, K, M, N,
P, Q, R, S, T, V, W, Y;

25 Position T 118 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, V, W, Y;

Position R 125 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, S, T, V, W, Y;

Position G 126 to A, C, D, E, F, H, I, K, L, M, N,
30 P, Q, R, S, T, V, W, Y;

Position R 128 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, S, T, V, W, Y;

Position L 129 to A, C, D, E, F, G, H, I, K, M, N,
P, Q, R, S, T, V, W, Y;

52. The allergen according to claims 51, wherein the allergen has at least 81%, preferably at least 85%, more preferably at least 90%, even more preferably at least 96%, most preferably at least 98% homology to SEQ ID NO 15.

53. The allergen according to claim 52, wherein the allergen has the amino acid sequence of SEQ ID NO 15.

54. The protein variant according to claim 30, wherein the allergen comprises one or more of the following substitutions corresponding to any of the following in SEQ ID NO 16:

Position	T	4	to	A, C, D, E, F, G, H, I, K, L, M,	
15	N, P, Q, R, S, V, W, Y;				
Position	D	28	to	A, C, E, F, G, H, I, K, L, M, N,	
	P, Q, R, S, T, V, W, Y;				
Position	V	31	to	A, C, D, E, F, G, H, I, K, L, M,	
	N, P, Q, R, S, T, W, Y;				
20	Position	Q	40	to	A, C, D, E, F, G, H, I, K, L, M,
	N, P, R, S, T, V, W, Y;				
Position	F	41	to	A, C, D, E, G, H, I, K, L, M, N,	
	P, Q, R, S, T, V, W, Y;				
Position	K	42	to	A, C, D, E, F, G, H, I, L, M, N,	
25	P, Q, R, S, T, V, W, Y;				
Position	D	44	to	A, C, E, F, G, H, I, K, L, M, N,	
	P, Q, R, S, T, V, W, Y;				
Position	E	45	to	A, C, D, F, G, H, I, K, L, M, N,	
	P, Q, R, S, T, V, W, Y;				
30	Position	A	47	to	C, D, E, F, G, H, I, K, L, M, N,
	P, Q, R, S, T, V, W, Y;				
Position	A	48	to	C, D, E, F, G, H, I, K, L, M, N,	
	P, Q, R, S, T, V, W, Y;				

390

Position K 51 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

Position D 54 to A, C, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

5 Position S 58 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, T, V, W, Y;

Position P 61 to A, C, D, E, F, G, H, I, K, L, M,
N, Q, R, S, T, V, W, Y;

Position T 62 to A, C, D, E, F, G, H, I, K, L, M,
10 N, P, Q, R, S, V, W, Y;

Position H 65 to A, C, D, E, F, G, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position G 68 to A, C, D, E, F, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

15 Position K 70 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

Position D 143 to A, C, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position G 146 to A, C, D, E, F, H, I, K, L, M, N,
20 P, Q, R, S, T, V, W, Y;

Position R 148 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, S, T, V, W, Y;

Position S 173 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, T, V, W, Y;

25 Position A 178 to C, D, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position K 181 to A, C, D, E, F, G, H, I, L, M,
N, P, Q, R, S, T, V, W, Y;

Position D 184 to A, C, E, F, G, H, I, K, L, M, N,
30 P, Q, R, S, T, V, W, Y;

Position E 185 to A, C, D, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position P 186 to A, C, D, E, F, G, H, I, K, L, M,
N, Q, R, S, T, V, W, Y;

391

Position G 187 to A, C, D, E, F, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position S 188 to A, C, D, E, F, G, H, I, K, L, M,
 N, P, Q, R, T, V, W, Y;
 5 Position A 190 to C, D, E, F, G, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position T 192 to A, C, D, E, F, G, H, I, K, L, M,
 N, P, Q, R, S, V, W, Y;
 Position V 203 to A, C, D, E, F, G, H, I, K, L, M,
 10 N, P, Q, R, S, T, W, Y;
 Position I 204 to A, C, D, E, F, G, H, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position E 207 to A, C, D, F, G, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 15 Position P 208 to A, C, D, E, F, G, H, I, K, L, M,
 N, Q, R, S, T, V, W, Y;
 Position G 209 to A, C, D, E, F, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position R 213 to A, C, D, E, F, G, H, I, K, L, M,
 20 N, P, Q, S, T, V, W, Y;
 Position K 215 to A, C, D, E, F, G, H, I, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position D 236 to A, C, E, F, G, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 25 Position P 238 to A, C, D, E, F, G, H, I, K, L, M,
 N, Q, R, S, T, V, W, Y;
 Position T 240 to A, C, D, E, F, G, H, I, K, L, M,
 N, P, Q, R, S, V, W, Y;
 Position P 241 to A, C, D, E, F, G, H, I, K, L, M,
 30 N, Q, R, S, T, V, W, Y;
 Position G 242 to A, C, D, E, F, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;

55. The allergen according to claims 54, wherein the allergen has at least 81%, preferably at least 85%, more preferably at least 90%, even more preferably at least 96%, most preferably at least 98% homology to SEQ ID NO 16.

5

56. The allergen according to claim 55, wherein the allergen has the amino acid sequence of SEQ ID NO 16.

57. The protein variant according to claim 30, wherein the al-
10 lergen comprises one or more of the following substitutions corresponding to any of the following in SEQ ID NO 17:

Position	A	33	to	C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
15 Position	A	36	to	C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
Position	T	38	to	A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W, Y;
Position	P	54	to	A, C, D, E, F, G, H, I, K, L, M, 20 N, Q, R, S, T, V, W, Y;
Position	R	56	to	A, C, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, Y;
Position	A	57	to	C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
25 Position	S	58	to	A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W, Y;
Position	V	68	to	A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, Y;
Position	L	70	to	A, C, D, E, F, G, H, I, K, M, N, 30 P, Q, R, S, T, V, W, Y;
Position	R	71	to	A, C, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, Y;
Position	Y	78	to	A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W;

393

Position K 80 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

Position K 81 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

5 Position S 83 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, T, V, W, Y;

Position A 84 to C, D, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position E 102 to A, C, D, F, G, H, I, K, L, M, N,
10 P, Q, R, S, T, V, W, Y;

Position K 103 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

Position P 106 to A, C, D, E, F, G, H, I, K, L, M,
N, Q, R, S, T, V, W, Y;

15 Position E 114 to A, C, D, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position D 118 to A, C, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position Y 119 to A, C, D, E, F, G, H, I, K, L, M,
20 N, P, Q, R, S, T, V, W;

Position I 121 to A, C, D, E, F, G, H, K, L, M, N,
P, Q, R, S, T, V, W, Y;

58. The allergen according to claims 57, wherein the allergen
25 has at least 81%, preferably at least 85%, more preferably at
least 90%, even more preferably at least 96%, most preferably at
least 98% homology to SEQ ID NO 17.

59. The allergen according to claim 58, wherein the allergen has
30 the amino acid sequence of SEQ ID NO 17.

60. The protein variant according to claim 30, wherein the al-
lergen comprises one or more of the following substitutions cor-
responding to any of the following in SEQ ID NO 18:

Position W 2 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, V, Y;

Position D 13 to A, C, E, F, G, H, I, K, L, M, N,
5 P, Q, R, S, T, V, W, Y;

Position E 15 to A, C, D, F, G, H, I, K, L, M,
N, P, Q, R, S, T, V, W, Y;

Position G 16 to A, C, D, E, F, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

10 Position D 28 to A, C, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position V 31 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, W, Y;

Position Q 34 to A, C, D, E, F, G, H, I, K, L, M,
15 N, P, R, S, T, V, W, Y;

Position Q 40 to A, C, D, E, F, G, H, I, K, L, M,
N, P, R, S, T, V, W, Y;

Position L 41 to A, C, D, E, F, G, H, I, K, M, N,
P, Q, R, S, T, V, W, Y;

20 Position P 43 to A, C, D, E, F, G, H, I, K, L, M,
N, Q, R, S, T, V, W, Y;

Position Q 44 to A, C, D, E, F, G, H, I, K, L, M,
N, P, R, S, T, V, W, Y;

Position D 47 to A, C, E, F, G, H, I, K, L, M, N,
25 P, Q, R, S, T, V, W, Y;

Position K 50 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

Position K 51 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

30 Position E 54 to A, C, D, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position G 57 to A, C, D, E, F, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

395

Position A 60 to C, D, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position T 62 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, V, W, Y;

5 Position G 67 to A, C, D, E, F, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position G 68 to A, C, D, E, F, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position E 69 to A, C, D, F, G, H, I, K, L, M, N,
10 P, Q, R, S, T, V, W, Y;

Position Y 71 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, V, W;

Position I 74 to A, C, D, E, F, G, H, K, L, M, N,
P, Q, R, S, T, V, W, Y;

15 Position Q 75 to A, C, D, E, F, G, H, I, K, L, M,
N, P, R, S, T, V, W, Y;

Position Q 78 to A, C, D, E, F, G, H, I, K, L, M,
N, P, R, S, T, V, W, Y;

Position R 83 to A, C, D, E, F, G, H, I, K, L, M,
20 N, P, Q, S, T, V, W, Y;

Position K 85 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

Position G 87 to A, C, D, E, F, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

25 Position P 88 to A, C, D, E, F, G, H, I, K, L, M,
N, Q, R, S, T, V, W, Y;

Position N 97 to A, C, D, E, F, G, H, I, K, L, M,
P, Q, R, S, T, V, W, Y;

Position D 106 to A, C, E, F, G, H, I, K, L, M, N,
30 P, Q, R, S, T, V, W, Y;

Position P 108 to A, C, D, E, F, G, H, I, K, L, M,
N, Q, R, S, T, V, W, Y;

Position T 110 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, V, W, Y;

396

Position R 120 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, S, T, V, W, Y;
Position D 123 to A, C, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;
5 Position Y 124 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, V, W;
Position E 127 to A, C, D, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;
Position E 129 to A, C, D, F, G, H, I, K, L, M, N,
10 P, Q, R, S, T, V, W, Y;

61. The allergen according to claims 60, wherein the allergen
has at least 81%, preferably at least 85%, more preferably at
least 90%, even more preferably at least 96%, most preferably at
15 least 98% homology to SEQ ID NO 18.

62. The allergen according to claim 61, wherein the allergen has
the amino acid sequence of SEQ ID NO 18.

20 63. The protein variant according to claim 30, wherein the al-
lergen comprises one or more of the following substitutions cor-
responding to any of the following in SEQ ID NO 19:

Position T 28 to A, C, D, E, F, G, H, I, K, L, M,
25 N, P, Q, R, S, V, W, Y;
Position T 31 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, V, W, Y;
Position A 33 to C, D, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;
30 Position G 34 to A, C, D, E, F, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;
Position A 36 to C, D, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position T 53 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, V, W, Y;

Position A 54 to C, D, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

5 Position R 56 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, S, T, V, W, Y;

Position G 64 to A, C, D, E, F, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position T 65 to A, C, D, E, F, G, H, I, K, L, M,
10 N, P, Q, R, S, V, W, Y;

Position R 66 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, S, T, V, W, Y;

Position V 68 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, W, Y;

15 Position R 71 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, S, T, V, W, Y;

Position D 74 to A, C, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position Y 78 to A, C, D, E, F, G, H, I, K, L, M,
20 N, P, Q, R, S, T, V, W;

Position S 83 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, T, V, W, Y;

Position A 84 to C, D, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

25 Position N 101 to A, C, D, E, F, G, H, I, K, L, M,
P, Q, R, S, T, V, W, Y;

Position E 102 to A, C, D, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position K 103 to A, C, D, E, F, G, H, I, L, M, N,
30 P, Q, R, S, T, V, W, Y;

Position Q 105 to A, C, D, E, F, G, H, I, K, L, M,
N, P, R, S, T, V, W, Y;

Position P 106 to A, C, D, E, F, G, H, I, K, L, M,
N, Q, R, S, T, V, W, Y;

398

Position T 108 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, V, W, Y;

Position K 115 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

5 Position Y 119 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, V, W;

Position T 133 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, V, W, Y;

Position V 136 to A, C, D, E, F, G, H, I, K, L, M,
10 N, P, Q, R, S, T, W, Y;

Position G 137 to A, C, D, E, F, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position D 150 to A, C, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

15 Position T 153 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, V, W, Y;

Position A 158 to C, D, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position A 161 to C, D, E, F, G, H, I, K, L, M, N,
20 P, Q, R, S, T, V, W, Y;

Position A 169 to C, D, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position K 175 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

25 Position D 176 to A, C, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position R 181 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, S, T, V, W, Y;

Position D 199 to A, C, E, F, G, H, I, K, L, M, N,
30 P, Q, R, S, T, V, W, Y;

Position R 200 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, S, T, V, W, Y;

Position K 206 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

399

Position G 207 to A, C, D, E, F, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position S 208 to A, C, D, E, F, G, H, I, K, L, M,
 N, P, Q, R, T, V, W, Y;
 5 Position A 209 to C, D, E, F, G, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position K 215 to A, C, D, E, F, G, H, I, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position E 227 to A, C, D, F, G, H, I, K, L, M, N,
 10 P, Q, R, S, T, V, W, Y;
 Position K 228 to A, C, D, E, F, G, H, I, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position I 229 to A, C, D, E, F, G, H, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 15 Position P 231 to A, C, D, E, F, G, H, I, K, L, M,
 N, Q, R, S, T, V, W, Y;
 Position G 232 to A, C, D, E, F, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position T 233 to A, C, D, E, F, G, H, I, K, L, M,
 20 N, P, Q, R, S, V, W, Y;
 Position N 236 to A, C, D, E, F, G, H, I, K, L, M,
 P, Q, R, S, T, V, W, Y;
 Position E 239 to A, C, D, F, G, H, I, K, L, M,
 N, P, Q, R, S, T, V, W, Y;
 25 Position D 243 to A, C, E, F, G, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position Y 244 to A, C, D, E, F, G, H, I, K, L, M,
 N, P, Q, R, S, T, V, W;
 Position I 246 to A, C, D, E, F, G, H, K, L, M, N,
 30 P, Q, R, S, T, V, W, Y;
 Position G 247 to A, C, D, E, F, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position Q 248 to A, C, D, E, F, G, H, I, K, L, M,
 N, P, R, S, T, V, W, Y;

400

Position G 249 to A, C, D, E, F, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

64. The allergen according to claims 63, wherein the allergen
5 has at least 81%, preferably at least 85%, more preferably at
least 90%, even more preferably at least 96%, most preferably at
least 98% homology to SEQ ID NO 19.

65. The allergen according to claim 64, wherein the allergen has
10 the amino acid sequence of SEQ ID NO 19.

66. The protein variant according to claim 30, wherein the al-
lergen comprises one or more of the following substitutions cor-
responding to any of the following in SEQ ID NO 20:

15

Position S 1 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, T, V, W, Y;

Position Y 5 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, V, W;

20 Position E 8 to A, C, D, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position H 9 to A, C, D, E, F, G, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

25 Position L 12 to A, C, D, E, F, G, H, I, K, M, N,
P, Q, R, S, T, V, W, Y;

Position E 47 to A, C, D, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position E 48 to A, C, D, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

30 Position E 70 to A, C, D, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position A 71 to C, D, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

401

Position R 76 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, S, T, V, W, Y;

Position K 78 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

5 Position G 80 to A, C, D, E, F, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position S 81 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, T, V, W, Y;

Position K 88 to A, C, D, E, F, G, H, I, L, M, N,
10 P, Q, R, S, T, V, W, Y;

Position G 90 to A, C, D, E, F, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position Q 91 to A, C, D, E, F, G, H, I, K, L, M,
N, P, R, S, T, V, W, Y;

15 Position E 99 to A, C, D, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position E 100 to A, C, D, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position P 101 to A, C, D, E, F, G, H, I, K, L, M,
20 N, Q, R, S, T, V, W, Y;

Position V 102 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, W, Y;

Position T 103 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, V, W, Y;

25 Position P 104 to A, C, D, E, F, G, H, I, K, L, M,
N, Q, R, S, T, V, W, Y;

Position G 105 to A, C, D, E, F, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position Q 106 to A, C, D, E, F, G, H, I, K, L, M,
30 N, P, R, S, T, V, W, Y;

Position E 112 to A, C, D, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position D 116 to A, C, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

402

Position Y 117 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, V, W;
Position I 119 to A, C, D, E, F, G, H, K, L, M, N,
P, Q, R, S, T, V, W, Y;
5 Position D 120 to A, C, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;
Position Q 121 to A, C, D, E, F, G, H, I, K, L, M,
N, P, R, S, T, V, W, Y;
Position G 122 to A, C, D, E, F, H, I, K, L, M, N,
10 P, Q, R, S, T, V, W, Y;
Position L 123 to A, C, D, E, F, G, H, I, K, M, N,
P, Q, R, S, T, V, W, Y;

67. The allergen according to claims 66, wherein the allergen
15 has at least 81%, preferably at least 85%, more preferably at
least 90%, even more preferably at least 96%, most preferably at
least 98% homology to SEQ ID NO 20.

68. The allergen according to claim 67, wherein the allergen has
20 the amino acid sequence of SEQ ID NO 20.

69. The protein variant according to claim 30, wherein the al-
lergen comprises one or more of the following substitutions cor-
responding to any of the following in SEQ ID NO 21:

25

Position L 4 to A, C, D, E, F, G, H, I, K, M, N,
P, Q, R, S, T, V, W, Y;
Position Y 6 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, V, W;
30 Position Y 17 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, V, W;
Position S 20 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, T, V, W, Y;

403

Position S 31 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, T, V, W, Y;

Position K 32 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

5 Position A 33 to C, D, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position K 37 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

10 70. The allergen according to claims 69, wherein the allergen
has at least 81%, preferably at least 85%, more preferably at
least 90%, even more preferably at least 96%, most preferably at
least 98% homology to SEQ ID NO 21.

15 71. The allergen according to claim 70, wherein the allergen has
the amino acid sequence of SEQ ID NO 21.

72. The protein variant according to claim 30, wherein the al-
lergen comprises one or more of the following substitutions cor-
20 responding to any of the following in SEQ ID NO 22:

Position N 6 to A, C, D, E, F, G, H, I, K, L, M,
P, Q, R, S, T, V, W, Y;

Position C 9 to A, D, E, F, G, H, I, K, L, M, N,
25 P, Q, R, S, T, V, W, Y;

Position K 23 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

Position Y 24 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, V, W;

30 Position G 25 to A, C, D, E, F, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position S 26 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, T, V, W, Y;

404

Position L 27 to A, C, D, E, F, G, H, I, K, M, N,
 P, Q, R, S, T, V, W, Y;
 Position K 28 to A, C, D, E, F, G, H, I, L, M, N,
 P, Q, R, S, T, V, W, Y;
 5 Position P 29 to A, C, D, E, F, G, H, I, K, L, M,
 N, Q, R, S, T, V, W, Y;
 Position K 34 to A, C, D, E, F, G, H, I, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position V 35 to A, C, D, E, F, G, H, I, K, L, M,
 10 N, P, Q, R, S, T, W, Y;
 Position Y 39 to A, C, D, E, F, G, H, I, K, L, M,
 N, P, Q, R, S, T, V, W;
 Position G 40 to A, C, D, E, F, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 15 Position L 41 to A, C, D, E, F, G, H, I, K, M, N,
 P, Q, R, S, T, V, W, Y;
 Position K 43 to A, C, D, E, F, G, H, I, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position E 45 to A, C, D, F, G, H, I, K, L, M, N,
 20 P, Q, R, S, T, V, W, Y;
 Position Q 47 to A, C, D, E, F, G, H, I, K, L, M,
 N, P, R, S, T, V, W, Y;
 Position D 48 to A, C, E, F, G, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 25 Position L 50 to A, C, D, E, F, G, H, I, K, M, N,
 P, Q, R, S, T, V, W, Y;
 Position K 51 to A, C, D, E, F, G, H, I, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position E 52 to A, C, D, F, G, H, I, K, L, M, N,
 30 P, Q, R, S, T, V, W, Y;
 Position D 55 to A, C, E, F, G, H, I, K, L, M, N,
 P, Q, R, S, T, V, W, Y;
 Position Q 58 to A, C, D, E, F, G, H, I, K, L, M,
 N, P, R, S, T, V, W, Y;

405

Position K 59 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

Position R 62 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, S, T, V, W, Y;

5 Position G 71 to A, C, D, E, F, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position P 72 to A, C, D, E, F, G, H, I, K, L, M,
N, Q, R, S, T, V, W, Y;

Position P 74 to A, C, D, E, F, G, H, I, K, L, M,
10 N, Q, R, S, T, V, W, Y;

Position P 75 to A, C, D, E, F, G, H, I, K, L, M,
N, Q, R, S, T, V, W, Y;

Position V 83 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, W, Y;

15 Position N 85 to A, C, D, E, F, G, H, I, K, L, M,
P, Q, R, S, T, V, W, Y;

Position D 86 to A, C, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position E 87 to A, C, D, F, G, H, I, K, L, M, N,
20 P, Q, R, S, T, V, W, Y;

Position Y 90 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, V, W;

Position Q 93 to A, C, D, E, F, G, H, I, K, L, M,
N, P, R, S, T, V, W, Y;

25 Position L 120 to A, C, D, E, F, G, H, I, K, M, N,
P, Q, R, S, T, V, W, Y;

Position T 121 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, V, W, Y;

Position G 122 to A, C, D, E, F, H, I, K, L, M, N,
30 P, Q, R, S, T, V, W, Y;

Position S 123 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, T, V, W, Y;

Position T 124 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, V, W, Y;

406

Position A 125 to C, D, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position A 126 to C, D, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

5 Position Y 128 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, V, W;

Position D 130 to A, C, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position D 140 to A, C, E, F, G, H, I, K, L, M, N,
10 P, Q, R, S, T, V, W, Y;

Position P 147 to A, C, D, E, F, G, H, I, K, L, M,
N, Q, R, S, T, V, W, Y;

Position K 148 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

15 Position K 150 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

Position S 152 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, T, V, W, Y;

Position G 153 to A, C, D, E, F, H, I, K, L, M, N,
20 P, Q, R, S, T, V, W, Y;

Position F 156 to A, C, D, E, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position K 158 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

25 Position H 161 to A, C, D, E, F, G, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position I 181 to A, C, D, E, F, G, H, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position E 183 to A, C, D, F, G, H, I, K, L, M, N,
30 P, Q, R, S, T, V, W, Y;

Position K 184 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

Position W 185 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, V, Y;

407

Position H 186 to A, C, D, E, F, G, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position N 199 to A, C, D, E, F, G, H, I, K, L, M,
P, Q, R, S, T, V, W, Y;

5 Position K 201 to A, C, D, E, F, G, H, I, L, M, N,
P, Q, R, S, T, V, W, Y;

Position N 202 to A, C, D, E, F, G, H, I, K, L, M,
P, Q, R, S, T, V, W, Y;

Position E 203 to A, C, D, F, G, H, I, K, L, M, N,
10 P, Q, R, S, T, V, W, Y;

Position E 204 to A, C, D, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, Y;

Position T 208 to A, C, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, V, W, Y;

15

73. The allergen according to claims 72, wherein the allergen has at least 81%, preferably at least 85%, more preferably at least 90%, even more preferably at least 96%, most preferably at least 98% homology to SEQ ID NO 22.

20

74. The allergen according to claim 73, wherein the allergen has the amino acid sequence of SEQ ID NO 22.

75. The protein variant according to claims 22-29, wherein the
25 protein variant is an enzyme.

76. The protein variant according to claim 75, wherein the enzyme is a protease, a lipolytic enzyme, a carbohydrase or a oxidoreductase.

30

77. The protein variant according to claim 76, wherein the protease is a subtilisin comprising one or more of the following substitutions corresponding to any of the following in SEQ ID NO 10:

Position -6 to A, C, D, E, F, G, H, I, K, L, M, N, P,
Q, R, S, T, V, W, Y, insertion, deletion;

Position -5 to A, C, D, E, F, G, H, I, K, L, M, N, P,
5 Q, R, S, T, V, W, Y, insertion, deletion;

Position -4 to A, C, D, E, F, G, H, I, K, L, M, N, P,
Q, R, S, T, V, W, Y, insertion, deletion;

Position -2 to A, C, D, E, F, G, H, I, K, L, M, N, P,
Q, R, S, T, V, W, Y, insertion, deletion;

10 Position 3a to A, C, D, E, F, G, H, I, K, L, M, N, P,
Q, R, S, T, V, W, Y, insertion, deletion;

Position 28a to A, C, D, E, F, G, H, I, K, L, M, N, P,
Q, R, S, T, V, W, Y, insertion, deletion;

Position 44a to A, C, D, E, F, G, H, I, K, L, M, N, P,
15 Q, R, S, T, V, W, Y, insertion, deletion;

Position 44b to A, C, D, E, F, G, H, I, K, L, M, N, P,
Q, R, S, T, V, W, Y, insertion, deletion;

Position 139 to A, C, D, E, F, G, H, I, K, L, M, N, P,
Q, R, S, T, V, W, Y, insertion, deletion;

20 Position 148 to A, C, D, E, F, G, H, I, K, L, M, N, P,
Q, R, S, T, V, W, Y, insertion, deletion;

Position 149 to A, C, D, E, F, G, H, I, K, L, M, N, P,
Q, R, S, T, V, W, Y, insertion, deletion;

Position 264a to A, C, D, E, F, G, H, I, K, L, M, N, P,
25 Q, R, S, T, V, W, Y, insertion, deletion;

78. The protein variant according to claim 76, wherein the pro-
tease is a subtilisin comprising one or more of the following
substitutions corresponding to any of the following in SEQ ID NO
30 10:

Position -1 to G, V, L, I, W, P, C, M, F, N, Q, Y, S,
T, D, E, R, H;

Position 1 to V, L, I, W, M, F, Y, S, T, R;

409

Position 2 to G, V, I, M, F, N, Q, Y, S, T, H;
 Position 3 to W, M, F, N, Q, Y, S, D, E, R, H;
 Position 4 to V, L, W, M, F, Y, R;
 Position 5 to V, L, I, W, M, F, N, Q, Y, T, R, H;
 5 Position 6 to G, V, L, I, W, P, M, N, Q, T, D, E, R,
 H;
 Position 9 to G, V, L, I, W, P, M, F, Q, Y, S, T, R,
 H;
 Position 10 to G, A, V, I, W, P, M, N, Q, Y, S, T, D,
 10 E, R;
 Position 12 to G, A, V, L, I, W, M, F, N, Q, Y, S, T,
 D, E;
 Position 14 to V, L, I, W, P, M, F, N, Q, Y, T, R, H;
 Position 15 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
 15 T, E, H;
 Position 17 to G, A, V, I, W, P, M, F, Y, H;
 Position 18 to G, A, L, I, W, P, M, F, N, Q, Y, T, D,
 E, H;
 Position 19 to A, V, I, W, M, F, N, Y, S, T, D, R, H;
 20 Position 20 to G, V, L, I, W, M, F, N, Q, Y, S, T, D,
 E;
 Position 21 to G, V, I, W, N, Q, Y, S, T, D, E, R, H;
 Position 22 to G, V, L, I, W, M, F, Y, S, T;
 Position 24 to G, V, L, I, W, M, F, N, Q, Y, S, D, E,
 25 R;
 Position 25 to G, A, V, L, I, W, M, F, N, Q, Y, S, T,
 D, E, R, H;
 Position 27 to G, L, I, W, P, M, F, Y, T, H;
 Position 38 to V, L, I, W, M, F, N, Q, Y, T, H;
 30 Position 39 to G, A, V, L, I, W, M, F, N, Q, Y, T, D,
 E, R, H;
 Position 40 to V, L, I, W, M, F, N, Q, Y, T, R, H;
 Position 42 to G, A, L, W, C, M, F, N, Q, Y, S, T, D,
 E, R, H;

410

Position 43 to G, L, H;
 Position 44 to G, V, L, I, W, P, M, F, Y, S, T;
 Position 45 to G, V, L, I, W, P, M, F, N, Q, Y, S, T,
 D, E, R, H;
 5 Position 46 to G, A, L, I, W, P, M, F, Y, H;
 Position 47 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
 T, D, E, R, H;
 Position 48 to A, L, I, P, M, F, N, Y, D, H;
 Position 49 to G, A, V, I, W, P, M, F, N, Q, Y, S, T,
 10 D, E, R, H;
 Position 50 to G, A, W, M, N, Q, Y, S, T, D, E, H;
 Position 51 to V, L, I, W, M, F, N, Y, R;
 Position 52 to V, L, I, W, M, F, Y, S, T, R;
 Position 53 to A, V, L, I, W, M, F, N, Q, Y, S, D, E,
 15 H;
 Position 54 to V, L, I, W, M, F, S, R;
 Position 55 to G, A, V, L, I, W, C, M, F, N, Q, Y, T,
 D, E, R, K, H;
 Position 56 to G, V, L, I, W, M, F, N, Q, Y, S, T, H;
 20 Position 57 to G, A, V, L, I, W, M, F, N, Q, Y, S, T,
 D, E, R, K, H;
 Position 58 to L, W, M, F, N, Y, R;
 Position 59 to A, V, L, I, C, T, H;
 Position 61 to V, L, I, W, M, F, Y;
 25 Position 62 to G, A, L, W, M, F, N, Y, R;
 Position 64 to G, V, L, I, W, P, C, M, F, N, Q, Y, S,
 T, D, E, R, K, H;
 Position 75 to L;
 Position 79 to I;
 30 Position 80 to G;
 Position 87 to A, V, L, I, W, M, F, Q, Y, S, T, D, E,
 H;
 Position 89 to G, V, L, I, W, P, F, N, Y, T, E;

411

Position 91 to G, A, V, L, I, W, P, M, N, Y, S, T, D,
 E, R, H;
 Position 98 to A;
 Position 99 to V, L, I, W, M, F, Q, Y, H;
 5 Position 100 to G, V, L, I, W, M, F, Y, R, H;
 Position 101 to V, I, W, M, F, N, Q, Y, H;
 Position 102 to V, L, I, W, M, F, Y, R, H, G;
 Position 108 to I;
 Position 109 to N;
 10 Position 112 to E;
 Position 113 to W;
 Position 115 to I;
 Position 117 to N;
 Position 118 to N;
 15 Position 126 to L;
 Position 127 to G, A, V, I, W, M, F, Y, R, H, L;
 Position 128 to I, W;
 Position 129 to W;
 Position 130 to W, F, Y, R;
 20 Position 131 to W, Y, R;
 Position 132 to L, W, M, F, Y, S, H;
 Position 133 to A, L, I, W, M, F, Y, R;
 Position 134 to L, I, W, F, N, Q, Y, R, H;
 Position 136 to G, A, W, P, N, Y, S, T, D, E, H;
 25 Position 137 to G, A, V, I, W, P, M, N, Y, H;
 Position 140 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
 T, H;
 Position 141 to G, V, L, I, W, P, M, F, Q, S, D, E, H;
 Position 143 to V, L, I, P, M, F, N, Y, R;
 30 Position 144 to L, W, P, M, F, N, Q, Y, S, D, E, R, H;
 Position 145 to G, V, L, I, W, M, F, Q, Y, D, E, R, H;
 Position 146 to G, A, W, L, I, W, M, F, N, Q, Y, T, D,
 E, R, H;
 Position 155 to V, L, I, W, M, F, Y, R;

412

Position 156 to V, I, W, F, R;
 Position 157 to G, A, V, L, I, W, M, F, Y, T, R, H;
 Position 158 to V, L, I, W, M, F, Y;
 Position 159 to A, W, M, Y, T, R, H;
 5 Position 160 to W, M, F, Y, R, H;
 Position 161 to I, W, M, F, Y, H;
 Position 167 to R, K;
 Position 171 to D;
 Position 172 to G, A, V, L, I, S, T, H;
 10 Position 173 to G, A, V, L, I, W, M, F, N, Q, Y, S, T,
 E, H;
 Position 181 to G, A, V, L, I, W, C, M, F, Q, Y, T, D,
 R, K, H;
 Position 182 to A, V, L, I, W, C, M, F, N, Q, Y, S, T,
 15 D, E, H;
 Position 183 to G, A, V, L, W, C, M, F, N, Q, Y, S, T,
 E, R, H;
 Position 184 to A, V, L, I, W, C, M, F, N, Q, Y, T, E,
 H;
 20 Position 185 to G, A, V, L, I, W, C, M, F, N, Q, Y, T,
 E, H;
 Position 186 to G, A, V, L, W, M, F, N, Q, Y, S, T, D,
 E, R, H;
 Position 188 to G, A, V, L, W, F, S, R, K;
 25 Position 189 to W, F;
 Position 191 to A, V, L, I, W, M, F, Y, T, R, H;
 Position 192 to G, L, I, W, M, N, Q, Y, S, T, D, R, H;
 Position 194 to W, N, Q, Y, D, H;
 Position 195 to W, P, Y;
 30 Position 196 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
 T, D, E, R, H;
 Position 203 to V, F, Y, R, H;
 Position 204 to I, W, M, Y, H;
 Position 206 to F;

413

Position 209 to Y, R;
 Position 210 to W, F, Y;
 Position 211 to L, W, M, F, Y, H;
 Position 212 to V, L, I, W, M, F, Y, T, R, H;
 5 Position 214 to W, Y, R;
 Position 215 to A, L, I, W, M, F, Y;
 Position 216 to A, L, I, W, M, F, Y, R;
 Position 217 to W, R;
 Position 218 to G, A, L, W, P, M, F, Y, R, H;
 10 Position 221 to S;
 Position 236 to S;
 Position 240 to N;
 Position 241 to W;
 Position 243 to N;
 15 Position 245 to Q;
 Position 247 to G, V, I, W, P, F, Y, S, T, R;
 Position 248 to W, P, F, Y, E, R, H;
 Position 249 to L, W, P, F, S, D, E, H;
 Position 251 to G, L, I, W, P, M, F, Y, H;
 20 Position 252 to G, A, W, P, N, Q, Y, T, E, R, H;
 Position 254 to G, V, L, I, W, M, F, N, Q, Y, S, D, E,
 R, H;
 Position 255 to G, L, W, M, F, N, Y, T, D, H;
 Position 256 to G, A, V, L, I, W, M, F, Q, Y, S, T, D,
 25 H;
 Position 257 to G, A, L, I, W, C, M, F, N, Q, Y, S, T,
 D, E, K, H;
 Position 258 to G, A, V, L, I, W, C, M, F, N, Q, Y, S,
 T, E, K, H;
 30 Position 259 to A, V, I, W, M, F, N, Q, Y, S, T, E, R;
 Position 260 to L, I, W, M, F, Y, T, H;
 Position 261 to L, N, S, H;
 Position 262 to G, A, V, L, I, W, P, F, N, Q, Y, T, D,
 E, R, H;

414

Position 263 to G, A, V, L, I, P, C, M, N, Q, Y, S, T,
R, K;
Position 265 to V, L, I, W, M, F, Y;
Position 269 to G, A, V, L, I, W, M, F, N, Q, Y, S, T,
5 E, R, H;
Position 271 to A, L, I, W, P, M, F, N, Y, S, T, R, H;
Position 272 to G, A, V, L, I, W, P, M, F, N, Q, Y, T,
D, E, H;
Position 275 to G, A, V, L, I, W, M, F, N, Y, T, D;

10

79. The protein variant according to claim 76, wherein the pro-
tease is a subtilisin comprising one or more of the following
substitutions corresponding to any of the following in SEQ ID NO

15 10:

Position -1 to Deletion;
Position 9 to Insertion, deletion;
Position 10 to Insertion, deletion;
20 Position 12 to Insertion, deletion;
Position 14 to Insertion, deletion;
Position 15 to Insertion, deletion;
Position 17 to Insertion, deletion;
Position 18 to Insertion, deletion;
25 Position 19 to Insertion, deletion;
Position 20 to Insertion, deletion;
Position 21 to Insertion, deletion;
Position 22 to Insertion, deletion;
Position 24 to Insertion, deletion;
30 Position 25 to Insertion, deletion;
Position 46 to Insertion, deletion;
Position 47 to Insertion, deletion;
Position 48 to Insertion, deletion;
Position 49 to Insertion, deletion;

415

	Position	50	to	Insertion, deletion;
	Position	51	to	Insertion, deletion;
	Position	52	to	Insertion, deletion;
	Position	53	to	Insertion, deletion;
5	Position	54	to	Insertion, deletion;
	Position	55	to	Insertion, deletion;
	Position	58	to	Insertion, deletion;
	Position	59	to	Insertion, deletion;
	Position	61	to	Insertion, deletion;
10	Position	64	to	Insertion, deletion;
	Position	78	to	Insertion;
	Position	80	to	Insertion;
	Position	91	to	Insertion, deletion;
	Position	98	to	Deletion;
15	Position	99	to	Deletion;
	Position	102	to	Deletion;
	Position	105	to	Insertion;
	Position	108	to	Insertion;
	Position	109	to	Insertion;
20	Position	112	to	Insertion;
	Position	113	to	Insertion;
	Position	115	to	Insertion;
	Position	116	to	Insertion;
	Position	117	to	Insertion;
25	Position	118	to	Insertion;
	Position	131	to	Deletion;
	Position	134	to	Insertion, deletion;
	Position	136	to	Insertion, deletion;
	Position	137	to	Insertion, deletion;
30	Position	140	to	Insertion, deletion;
	Position	141	to	Insertion, deletion;
	Position	143	to	Insertion, deletion;
	Position	144	to	Insertion, deletion;
	Position	145	to	Insertion, deletion;

416

	Position	146	to	Insertion, deletion;
	Position	171	to	Deletion;
	Position	172	to	Deletion;
	Position	173	to	Deletion;
5	Position	181	to	Deletion;
	Position	182	to	Deletion;
	Position	183	to	Deletion;
	Position	184	to	Deletion;
	Position	185	to	Deletion;
10	Position	186	to	Deletion;
	Position	188	to	Deletion;
	Position	189	to	Deletion;
	Position	191	to	Deletion;
	Position	192	to	Deletion;
15	Position	195	to	Deletion;
	Position	196	to	Insertion, deletion;
	Position	221	to	Insertion;
	Position	236	to	Insertion;
	Position	237	to	Insertion;
20	Position	238	to	Insertion;
	Position	239	to	Insertion;
	Position	240	to	Insertion;
	Position	241	to	Insertion;
	Position	242	to	Insertion;
25	Position	243	to	Insertion;
	Position	244	to	Insertion;
	Position	245	to	Insertion;
	Position	247	to	Insertion, deletion;
	Position	248	to	Insertion, deletion;
30	Position	249	to	Insertion, deletion;
	Position	251	to	Insertion, deletion;
	Position	252	to	Insertion, deletion;
	Position	254	to	Insertion, deletion;
	Position	255	to	Insertion, deletion;

417

Position 256 to Insertion, deletion;
 Position 257 to Insertion, deletion;
 Position 258 to Insertion, deletion;
 Position 259 to Insertion, deletion;
 5 Position 260 to Insertion, deletion;
 Position 261 to Insertion, deletion;
 Position 262 to Insertion, deletion;
 Position 263 to Insertion, deletion;
 Position 265 to Insertion, deletion;
 10 Position 269 to Insertion, deletion;
 Position 271 to Insertion, deletion;
 Position 272 to Insertion, deletion;
 Position 275 to Insertion, deletion;

15

80. The protein variant according to claim 76, wherein the protease is a subtilisin comprising one or more of the following substitutions corresponding to any of the following in SEQ ID NO 10:

20

Position 7 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, H;
 Position 8 to G, A, L, W, P, C, M, F, N, Q, Y, S, T, D, E, R, K, H;
 25 Position 13 to G, L, I, W, P, M, F, N, Q, Y, S, D, E, H;
 Position 16 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, D, E, R, H;
 Position 23 to G, A, V, L, I, W, M, F, Y, E, R, H;
 30 Position 26 to G, A, V, L, I, W, M, F, N, Q, Y, S, T, D, E, R, H;
 Position 28 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, K, H;

418

Position 29 to G, A, V, L, I, W, P, M, F, N, Q, Y, S;
 T, D, E, R, K, H;
 Position 33 to V, L, I, W, C, M, F, N, Q, Y, R, H;
 Position 35 to G, A, V, L, I, W, M, F, N, Q, Y, S, T,
 5 D, E, R, K, H;
 Position 36 to V, L, I, W, P, M, F, N, Y, S, T, R, H;
 Position 37 to L, I, W, M, F, N, Q, Y, S, R, H;
 Position 41 to G, V, L, I, W, M, F, N, Q, Y, S, T, R,
 H;
 10 Position 60 to G, A, V, L, I, W, C, M, F, Q, Y, T, D,
 R, K, H;
 Position 63 to G, A, V, L, I, W, M, F, Y, T, R, H;
 Position 73 to A;
 Position 74 to A;
 15 Position 81 to V;
 Position 82 to L;
 Position 86 to G, A, V, L, I, W, M, F, N, Q, Y, T, D,
 E, R, H;
 Position 88 to A, V, L, I, W, M, F, N, Q, Y, S, T, D,
 20 E, R, H;
 Position 92 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
 T, D, E, R, K, H;
 Position 93 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
 T, D, E, R, K, H;
 25 Position 94 to G, V, L, I, W, P, M, F, N, Y, T, D, E,
 K, H;
 Position 96 to L, W, F, Y, R, K;
 Position 97 to V, L, W, C, M, F, Y, H;
 Position 111 to I;
 30 Position 114 to A;
 Position 119 to M;
 Position 124 to M;
 Position 135 to G, L, P, C, N, Q, T, R, H;

419

Position 138 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
T, D, E, R, H;

Position 142 to G, A, L, I, W, P, C, M, F, N, Q, Y, S,
T, D, E, R, K, H;

5 Position 147 to G, A, V, L, W, M, F, N, Q, Y, S, T, D,
E, R, K, H;

Position 151 to G, V, L, I, W, P, C, M, F, N, Q, Y, S,
T, D, E, R, K, H;

Position 162 to I, W, F, Y, R;

10 Position 163 to V, W, M, F, H;

Position 168 to G, V, L, I, W, C, M, F, N, Q, Y, S, T,
D, E, R, K, H;

Position 169 to C, E, F, G, H, I, K, L, M, N, Q, R, T,
V, W, Y;

15 Position 174 to G, A, L, I, W, P, C, M, F, N, Q, Y, S,
T, D, E, R, K, H;

Position 176 to G, A, V, L, I, W, P, C, M, F, N, Q, Y,
S, T, D, E, R, K, H;

Position 179 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
20 T, D, E, R, K, H;

Position 187 to A, V, L, I, W, M, F, Y, R;

Position 190 to G, A, V, L, I, W, C, M, F, N, Q, Y, S,
T, R, K, H;

Position 193 to G, V, L, I, W, M, F, N, Q, Y, S, T, D,
25 E, R, H;

Position 197 to G, V, L, I, W, P, M, F, Q, Y, S, T, H;

Position 198 to G, A, L, I, W, P, C, M, F, N, Q, Y, S,
T, D, E, R, K, H;

Position 205 to W, F, Y, R, K;

30 Position 208 to A, V, L, I, W, C, M, F, Y, T, R, K, H;

Position 219 to G, A, V, L, I, W, F, Y, R, H;

Position 222 to M;

Position 232 to A;

Position 233 to L;

420

Position 234 to I;
 Position 250 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
 T, D, E, R, H;
 Position 267 to G, A, V, L, I, W, M, F, N, Q, Y, S, T,
 5 D, E, R, H;
 Position 268 to G, V, L, I, W, C, M, N, Q, Y, S, T, D,
 E, R, K, H;
 Position 270 to G, L, I, W, P, M, F, N, Q, Y, S, T, D,
 E, R, K, H;
 10 Position 273 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
 T, D, E, R, K, H;
 Position 274 to W, P, M, F, N, Q, Y, T, D, E, R, H;

81. The protein variant according to claim 76, wherein the pro-
 15 tease is a subtilisin comprising one or more of the following
 substitutions corresponding to any of the following in SEQ ID NO
 10:

Position 13 to Insertion, deletion;
 20 Position 16 to Insertion, deletion;
 Position 23 to Insertion, deletion;
 Position 26 to Insertion, deletion;
 Position 28 to Insertion, deletion;
 Position 29 to Insertion, deletion;
 25 Position 35 to Deletion;
 Position 60 to Insertion, deletion;
 Position 63 to Insertion;
 Position 81 to Insertion;
 Position 82 to Insertion;
 30 Position 92 to Insertion, deletion;
 Position 93 to Insertion, deletion;
 Position 94 to Insertion, deletion;
 Position 96 to Deletion,
 Position 106 to Insertion,

421

	Position	111	to	Insertion,
	Position	114	to	Insertion,
	Position	119	to	Insertion,
	Position	124	to	Insertion,
5	Position	138	to	Insertion, deletion;
	Position	142	to	Insertion, deletion;
	Position	147	to	Insertion, deletion;
	Position	151	to	Insertion, deletion;
	Position	174	to	Insertion, deletion;
10	Position	176	to	Insertion, deletion;
	Position	179	to	Insertion, deletion;
	Position	187	to	Deletion;
	Position	190	to	Deletion;
	Position	193	to	Deletion;
15	Position	197	to	Insertion, deletion;
	Position	198	to	Insertion, deletion;
	Position	232	to	Insertion,
	Position	233	to	Insertion,
	Position	234	to	Insertion,
20	Position	246	to	Insertion,
	Position	250	to	Insertion, deletion;
	Position	267	to	Insertion, deletion;
	Position	268	to	Insertion, deletion;
	Position	270	to	Insertion, deletion;
25	Position	273	to	Insertion, deletion;

82. The protein variant according to claims 76-81, wherein the protease is a savinase-like subtilisin comprising one or more
30 of the following substitutions corresponding to any of the following in SEQ ID NO: 10:

Position	2	to	G, V, I, M, F, N, Q, Y, S, T, H,
Position	3	to	W, M, F, N, Q, Y, S, D, E, R, H,

422

Position 4 to V, L, W, M, F, Y, R,
Position 6 to G, V, L, I, W, P, M, N, Q, T, D, E, R,
H,
Position 9 to G, V, L, I, W, P, M, F, Q, Y, S, T, R,
5 H, insertion, deletion,
Position 10 to G, A, V, I, W, P, M, N, Q, Y, S, T, D,
E, R, insertion, deletion,
Position 12 to G, A, V, L, I, W, M, F, N, Q, Y, S, T,
D, E, insertion, deletion,
10 Position 14 to V, L, I, W, P, M, F, N, Q, Y, T, R, H,
insertion, deletion,
Position 15 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
T, E, H, insertion, deletion,
Position 17 to G, A, V, I, W, P, M, F, Y, H, insertion,
15 deletion,
Position 18 to G, A, L, I, W, P, M, F, N, Q, Y, T, D,
E, H, insertion, deletion,
Position 19 to A, V, I, W, M, F, N, Y, S, T, D, R, H,
insertion, deletion,
20 Position 20 to G, V, L, I, W, M, F, N, Q, Y, S, T, D,
E, insertion, deletion,
Position 21 to G, V, I, W, N, Q, Y, S, T, D, E, R, H,
insertion, deletion,
Position 22 to G, V, L, I, W, M, F, Y, S, T, insertion,
25 deletion,
Position 24 to G, V, L, I, W, M, F, N, Q, Y, S, D, E,
R, insertion, deletion,
Position 25 to G, A, V, L, I, W, M, F, N, Q, Y, S, T,
D, E, R, H, insertion, deletion,
30 Position 27 to G, L, I, W, P, M, F, Y, T, H,
Position 37 to L, I, W, M, F, N, Q, Y, S, R, H,
Position 40 to V, L, I, W, M, F, N, Q, Y, T, R, H,
Position 42 to G, A, L, W, C, M, F, N, Q, Y, S, T, D,
E, R, H,

423

Position 43 to G, L, H,
Position 44 to G, V, L, I, W, P, M, F, Y, S, T,
Position 45 to G, V, L, I, W, P, M, F, N, Q, Y, S, T,
D, E, R, H,
5 Position 46 to G, A, L, I, W, P, M, F, Y, H, insertion,
deletion,
Position 47 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
T, D, E, R, H, insertion, deletion,
Position 48 to A, L, I, P, M, F, N, Y, D, H, insertion,
10 deletion,
Position 50 to G, A, W, M, N, Q, Y, S, T, D, E, H, in-
sertion, deletion,
Position 51 to V, L, I, W, M, F, N, Y, R, deletion, in-
sertion,
15 Position 54 to V, L, I, W, M, F, S, R, deletion, inser-
tion,
Position 55 to G, A, V, L, I, W, C, M, F, N, Q, Y, T,
D, E, R, K, H, deletion, insertion,
Position 57 to G, A, V, L, I, W, M, F, N, Q, Y, S, T,
20 D, E, R, K, H,
Position 58 to L, W, M, F, N, Y, R, insertion, dele-
tion,
Position 59 to A, V, L, I, C, T, H, insertion, dele-
tion,
25 Position 61 to V, L, I, W, M, F, Y, insertion, dele-
tion,
Position 64 to G, V, L, I, W, P, C, M, F, N, Q, Y, S,
T, D, E, R, K, H, insertion, deletion,
Position 75 to L,
30 Position 78 to insertion,
Position 79 to I,
Position 87 to A, V, L, I, W, M, F, Q, Y, S, T, D, E,
H,
Position 89 to G, V, L, I, W, P, F, N, Y, T, E,

424

Position 91 to G, A, V, L, I, W, P, M, N, Y, S, T, D,
 E, R, H, insertion, deletion,
 Position 98 to A, deletion,
 Position 100 to G, V, L, I, W, M, F, Y, R, H,
 5 Position 101 to V, I, W, M, F, N, Q, Y, H,
 Position 102 to V, L, I, W, M, F, Y, R, H, G, deletion,
 Position 109 to N, insertion,
 Position 112 to E, insertion,
 Position 113 to W, insertion,
 10 Position 116 to insertion,
 Position 117 to N, insertion,
 Position 126 to L,
 Position 127 to G, A, V, I, W, M, F, Y, R, H, L,
 Position 128 to I, W,
 15 Position 129 to W,
 Position 130 to W, F, Y, R,
 Position 131 to W, Y, R, deletion,
 Position 132 to L, W, M, F, Y, S, H,
 Position 133 to A, L, I, W, M, F, Y, R,
 20 Position 134 to L, I, W, F, N, Q, Y, R, H, insertion,
 deletion,
 Position 136 to G, A, W, P, N, Y, S, T, D, E, H, inser-
 tion, deletion,
 Position 137 to G, A, V, I, W, P, M, N, Y, H, insertion,
 25 deletion,
 Position 140 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
 T, H, insertion, deletion,
 Position 141 to G, V, L, I, W, P, M, F, Q, S, D, E, H,
 insertion, deletion,
 30 Position 143 to V, L, I, P, M, F, N, Y, R, insertion,
 deletion,
 Position 144 to L, W, P, M, F, N, Q, Y, S, D, E, R, H,
 insertion, deletion,

425

Position 145 to G, V, L, I, W, M, F, Q, Y, D, E, R, H,
insertion, deletion,

Position 146 to G, A, W, L, I, W, M, F, N, Q, Y, T, D,
E, R, H, insertion, deletion,

5 Position 155 to V, L, I, W, M, F, Y, R,
Position 156 to V, I, W, F, R,
Position 157 to G, A, V, L, I, W, M, F, Y, T, R, H,
Position 158 to V, L, I, W, M, F, Y,
Position 160 to W, M, F, Y, R, H,

10 Position 161 to I, W, M, F, Y, H,
Position 167 to R, K,
Position 170 to A, C, D, E, F, G, H, I, K, L, M, N, P,
Q, R, S, T, V, W, Y;
Position 171 to D, deletion,

15 Position 172 to G, A, V, L, I, S, T, H, deletion,
Position 173 to G, A, V, L, I, W, M, F, N, Q, Y, S, T,
E, H, deletion,
Position 181 to G, A, V, L, I, W, C, M, F, Q, Y, T, D,
R, K, H, deletion,

20 Position 183 to G, A, V, L, W, C, M, F, N, Q, Y, S, T,
E, R, H, deletion,
Position 184 to A, V, L, I, W, C, M, F, N, Q, Y, T, E,
H, deletion,
Position 185 to G, A, V, L, I, W, C, M, F, N, Q, Y, T,
25 E, H, deletion,
Position 186 to G, A, V, L, W, M, F, N, Q, Y, S, T, D,
E, R, H, deletion,
Position 188 to G, A, V, L, W, F, S, R, K, deletion,
Position 189 to W, F, deletion,

30 Position 191 to A, V, L, I, W, M, F, Y, T, R, H, dele-
tion,
Position 192 to G, L, I, W, M, N, Q, Y, S, T, D, R, H,
deletion,
Position 194 to W, N, Q, Y, D, H,

426

Position 195 to W, P, Y, deletion,
Position 197 to G, V, L, I, W, P, M, F, Q, Y, S, T, H,
insertion, deletion,
Position 203 to V, F, Y, R, H,
5 Position 206 to F,
Position 209 to Y, R,
Position 210 to W, F, Y,
Position 212 to V, L, I, W, M, F, Y, T, R, H,
Position 214 to W, Y, R,
10 Position 216 to A, L, I, W, M, F, Y, R,
Position 217 to W, R,
Position 218 to G, A, L, W, P, M, F, Y, R, H,
Position 221 to S, insertion,
Position 236 to S, insertion,
15 Position 237 to insertion,
Position 239 to insertion,
Position 240 to N, insertion,
Position 241 to W, insertion,
Position 242 to insertion,
20 Position 244 to insertion,
Position 245 to Q, insertion,
Position 247 to G, V, I, W, P, F, Y, S, T, R, insertion,
deletion,
Position 248 to W, P, F, Y, E, R, H, insertion, dele-
25 tion,
Position 251 to G, L, I, W, P, M, F, Y, H, insertion,
deletion,
Position 252 to G, A, W, P, N, Q, Y, T, E, R, H, inser-
tion, deletion,
30 Position 255 to G, L, W, M, F, N, Y, T, D, H, insertion,
deletion,
Position 256 to G, A, V, L, I, W, M, F, Q, Y, S, T, D,
H, insertion, deletion,

427

Position 257 to G, A, L, I, W, C, M, F, N, Q, Y, S, T,
D, E, K, H, insertion, deletion,
Position 258 to G, A, V, L, I, W, C, M, F, N, Q, Y, S,
T, E, K, H, insertion, deletion,
5 Position 259 to A, V, I, W, M, F, N, Q, Y, S, T, E, R,
insertion, deletion,
Position 260 to L, I, W, M, F, Y, T, H, insertion, dele-
tion,
Position 261 to L, N, S, H, insertion, deletion,
10 Position 262 to G, A, V, L, I, W, P, F, N, Q, Y, T, D,
E, R, H, insertion, deletion,
Position 263 to G, A, V, L, I, P, C, M, N, Q, Y, S, T,
R, K, insertion, deletion,
Position 265 to V, L, I, W, M, F, Y, insertion, dele-
15 tion,
Position 271 to A, L, I, W, P, M, F, N, Y, S, T, R, H,
insertion, deletion,
Position 272 to G, A, V, L, I, W, P, M, F, N, Q, Y, T,
D, E, H, insertion, deletion,
20 Position 275 to G, A, V, L, I, W, M, F, N, Y, T, D, in-
sertion, deletion,

83. The protein variant according to claim 82, wherein the
savinase-like subtilisin comprises one or more of the following
25 substitutions corresponding to any of the following in SEQ ID
NO: 10:

Position 6 to G, V, L, I, W, P, M, N, Q, T, D, E, R,
H,
30 Position 9 to G, V, L, I, W, P, M, F, Q, Y, S, T, R,
H, insertion, deletion,
Position 10 to G, A, V, I, W, P, M, N, Q, Y, S, T, D,
E, R, insertion, deletion,

428

Position 14 to V, L, I, W, P, M, F, N, Q, Y, T, R, H,
insertion, deletion,
Position 15 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
T, E, H, insertion, deletion,
5 Position 17 to G, A, V, I, W, P, M, F, Y, H, insertion,
deletion,
Position 18 to G, A, L, I, W, P, M, F, N, Q, Y, T, D,
E, H, insertion, deletion,
Position 19 to A, V, I, W, M, F, N, Y, S, T, D, R, H,
10 insertion, deletion,
Position 20 to G, V, L, I, W, M, F, N, Q, Y, S, T, D,
E, insertion, deletion,
Position 21 to G, V, I, W, N, Q, Y, S, T, D, E, R, H,
insertion, deletion,
15 Position 37 to L, I, W, M, F, N, Q, Y, S, R, H,
Position 43 to G, L, H,
Position 45 to G, V, L, I, W, P, M, F, N, Q, Y, S, T,
D, E, R, H,
Position 47 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
20 T, D, E, R, H, insertion, deletion,
Position 50 to G, A, W, M, N, Q, Y, S, T, D, E, H, in-
sertion, deletion,
Position 51 to V, L, I, W, M, F, N, Y, R, deletion, in-
sertion,
25 Position 54 to V, L, I, W, M, F, S, R, deletion, inser-
tion,
Position 59 to A, V, L, I, C, T, H, insertion, dele-
tion,
Position 89 to G, V, L, I, W, P, F, N, Y, T, E,
30 Position 91 to G, A, V, L, I, W, P, M, N, Y, S, T, D,
E, R, H, insertion, deletion,
Position 101 to V, I, W, M, F, N, Q, Y, H,
Position 109 to N, insertion,
Position 112 to E, insertion,

429

Position 113 to W, insertion,
Position 127 to G, A, V, I, W, M, F, Y, R, H, L,
Position 128 to I, W,
Position 129 to W,
5 Position 130 to W, F, Y, R,
Position 131 to W, Y, R, deletion,
Position 133 to A, L, I, W, M, F, Y, R,
Position 136 to G, A, W, P, N, Y, S, T, D, E, H, inser-
tion, deletion,
10 Position 137 to G, A, V, I, W, P, M, N, Y, H, insertion,
deletion,
Position 140 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
T, H, insertion, deletion,
Position 141 to G, V, L, I, W, P, M, F, Q, S, D, E, H,
15 insertion, deletion,
Position 143 to V, L, I, P, M, F, N, Y, R, insertion,
deletion,
Position 144 to L, W, P, M, F, N, Q, Y, S, D, E, R, H,
insertion, deletion,
20 Position 145 to G, V, L, I, W, M, F, Q, Y, D, E, R, H,
insertion, deletion,
Position 146 to G, A, W, L, I, W, M, F, N, Q, Y, T, D,
E, R, H, insertion, deletion,
Position 155 to V, L, I, W, M, F, Y, R,
25 Position 157 to G, A, V, L, I, W, M, F, Y, T, R, H,
Position 158 to V, L, I, W, M, F, Y,
Position 160 to W, M, F, Y, R, H,
Position 161 to I, W, M, F, Y, H,
Position 167 to R, K,
30 Position 170 to A, C, D, E, F, G, H, I, K, L, M, N, P,
Q, R, S, T, V, W, Y;
Position 171 to D, deletion,
Position 172 to G, A, V, L, I, S, T, H, deletion,

430

Position 173 to G, A, V, L, I, W, M, F, N, Q, Y, S, T,
E, H, deletion,
Position 181 to G, A, V, L, I, W, C, M, F, Q, Y, T, D,
R, K, H, deletion,
5 Position 184 to A, V, L, I, W, C, M, F, N, Q, Y, T, E,
H, deletion,
Position 185 to G, A, V, L, I, W, C, M, F, N, Q, Y, T,
E, H, deletion,
Position 186 to G, A, V, L, W, M, F, N, Q, Y, S, T, D,
10 E, R, H, deletion,
Position 188 to G, A, V, L, W, F, S, R, K, deletion,
Position 189 to W, F, deletion,
Position 192 to G, L, I, W, M, N, Q, Y, S, T, D, R, H,
deletion,
15 Position 194 to W, N, Q, Y, D, H,
Position 195 to W, P, Y, deletion,
Position 197 to G, V, L, I, W, P, M, F, Q, Y, S, T, H,
insertion, deletion,
Position 203 to V, F, Y, R, H,
20 Position 210 to W, F, Y,
Position 218 to G, A, L, W, P, M, F, Y, R, H,
Position 236 to S, insertion,
Position 237 to insertion,
Position 239 to insertion,
25 Position 240 to N, insertion,
Position 241 to W, insertion,
Position 242 to insertion,
Position 244 to insertion,
Position 245 to Q, insertion,
30 Position 247 to G, V, I, W, P, F, Y, S, T, R, insertion,
deletion,
Position 251 to G, L, I, W, P, M, F, Y, H, insertion,
deletion,

431

Position 255 to G, L, W, M, F, N, Y, T, D, H, insertion,
deletion,

Position 256 to G, A, V, L, I, W, M, F, Q, Y, S, T, D,
H, insertion, deletion,

5 Position 257 to G, A, L, I, W, C, M, F, N, Q, Y, S, T,
D, E, K, H, insertion, deletion,

Position 258 to G, A, V, L, I, W, C, M, F, N, Q, Y, S,
T, E, K, H, insertion, deletion,

Position 260 to L, I, W, M, F, Y, T, H, insertion, dele-
10 tion,

Position 262 to G, A, V, L, I, W, P, F, N, Q, Y, T, D,
E, R, H, insertion, deletion,

Position 265 to V, L, I, W, M, F, Y, insertion, dele-
tion,

15 Position 271 to A, L, I, W, P, M, F, N, Y, S, T, R, H,
insertion, deletion,

Position 272 to G, A, V, L, I, W, P, M, F, N, Q, Y, T,
D, E, H, insertion, deletion,

Position 275 to G, A, V, L, I, W, M, F, N, Y, T, D, in-
20 sertion, deletion,

84. The savinase-like subtilisin according to claims 82-83, whe-
rein the subtilisin has at least 81%, preferably at least 96%,
more preferably at least 98%, most preferably at least 99%
25 homology to SEQ ID NO 24.

85. The savinase-like subtilisin according to claim 84, wherein
the subtilisin has any of the amino acid sequence of SEQ ID NO
24, 26, 27, 28, 29, 30, 31, 32, 34, 35.

30

86. The protein variant according to claims 76-81, wherein the
protease is a savinase-like subtilisin comprising one or more
of the following substitutions corresponding to any of the fol-
lowing in SEQ ID NO: 10:

432

Position 8 to G, A, L, W, P, C, M, F, N, Q, Y, S, T,
D, E, R, K, H,
Position 16 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
5 D, E, R, H, insertion, deletion,
Position 23 to G, A, V, L, I, W, M, F, Y, E, R, H, in-
sertion, deletion,
Position 26 to G, A, V, L, I, W, M, F, N, Q, Y, S, T,
D, E, R, H, insertion, deletion,
10 Position 35 to G, A, V, L, I, W, M, F, N, Q, Y, S, T,
D, E, R, K, H, deletion,
Position 38 to V, L, I, W, M, F, N, Q, Y, T, H,
Position 39 to G, A, V, L, I, W, M, F, N, Q, Y, T, D,
E, R, H,
15 Position 41 to G, V, L, I, W, M, F, N, Q, Y, S, T, R,
H,
Position 60 to G, A, V, L, I, W, C, M, F, Q, Y, T, D,
R, K, H, insertion, deletion,
Position 73 to A,
20 Position 74 to A,
Position 80 to G, insertion,
Position 81 to V, insertion,
Position 86 to G, A, V, L, I, W, M, F, N, Q, Y, T, D,
E, R, H,
25 Position 88 to A, V, L, I, W, M, F, N, Q, Y, S, T, D,
E, R, H,
Position 90 to A, C, D, E, F, G, H, I, K, L, M, N, P,
Q, R, S, T, V, W, Y, insertion, deletion,
Position 93 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
30 T, D, E, R, K, H, insertion, deletion,
Position 108 to I, insertion,
Position 111 to I, insertion,
Position 124 to M, insertion,
Position 135 to G, L, P, C, N, Q, T, R, H,

433

Position 142 to G, A, L, I, W, P, C, M, F, N, Q, Y, S,
T, D, E, R, K, H, insertion, deletion,
Position 147 to G, A, V, L, W, M, F, N, Q, Y, S, T, D,
E, R, K, H, insertion, deletion,
5 Position 148 to G, A, V, L, I, W, P, C, M, F, N, Q, Y,
S, T, D, E, R, K, H, insertion, deletion,
Position 149 to G, A, V, L, I, W, P, C, M, F, N, Q, Y,
S, T, D, E, R, K, H, insertion, deletion,
Position 151 to G, V, L, I, W, P, C, M, F, N, Q, Y, S,
10 T, D, E, R, K, H, insertion, deletion,
Position 163 to V, W, M, F, H,
Position 168 to G, V, L, I, W, C, M, F, N, Q, Y, S, T,
D, E, R, K, H,
Position 169 to C, E, F, G, H, I, K, L, M, N, Q, R, T,
15 V, W, Y,
Position 174 to G, A, L, I, W, P, C, M, F, N, Q, Y, S,
T, D, E, R, K, H, insertion, deletion,
Position 179 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
T, D, E, R, K, H, insertion, deletion,
20 Position 190 to G, A, V, L, I, W, C, M, F, N, Q, Y, S,
T, R, K, H, deletion,
Position 193 to G, V, L, I, W, M, F, N, Q, Y, S, T, D,
E, R, H, deletion,
Position 196 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
25 T, D, E, R, H, insertion, deletion,
Position 208 to A, V, L, I, W, C, M, F, Y, T, R, K, H,
Position 213 to N, oN, E,
Position 215 to A, L, I, W, M, F, Y,
Position 232 to A, insertion,
30 Position 233 to L, insertion,
Position 234 to I, insertion,
Position 246 to insertion,
Position 250 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
T, D, E, R, H, insertion, deletion,

434

Position 254 to G, V, L, I, W, M, F, N, Q, Y, S, D, E,
R, H, insertion, deletion,

Position 267 to G, A, V, L, I, W, M, F, N, Q, Y, S, T,
D, E, R, H, insertion, deletion,

5 Position 268 to G, V, L, I, W, C, M, N, Q, Y, S, T, D,
E, R, K, H, insertion, deletion,

Position 269 to G, A, V, L, I, W, M, F, N, Q, Y, S, T,
E, R, H, insertion, deletion,

Position 273 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
10 T, D, E, R, K, H, insertion, deletion,

87. The savinase-like subtilisin according to claim 86, wherein
the subtilisin has at least 81%, preferably at least 96%, more
preferably at least 98%, most preferably at least 99% homology
15 to SEQ ID NO 24.

88. The savinase-like subtilisin according to claim 87, wherein
the subtilisin has any of the amino acid sequence of SEQ ID NO
24, 26, 27, 28, 29, 30, 31, 32, 34, 35.

20

89. The protein variant according to claims 76-81 having modi-
fied immunogenicity as compared to its parent protein having at
least 81% homology to SEQ ID NO 25 comprising one or more of the
following substitutions corresponding to any of the following in
25 SEQ ID NO 25:

Position 21 to G, V, I, W, N, Q, Y, S, T, D, E, R, H,
insertion, deletion,

Position 27 to G, L, I, W, P, M, F, Y, T, H,

30 Position 50 to G, A, W, M, N, Q, Y, S, T, D, E, H, in-
sertion, deletion,

Position 52 to V, L, I, W, M, F, Y, S, T, R, deletion,
insertion,

435

Position 55 to G, A, V, L, I, W, C, M, F, N, Q, Y, T,
D, E, R, K, H, deletion, insertion,
Position 129 to W,
Position 133 to A, L, I, W, M, F, Y, R,
5 Position 172 to G, A, V, L, I, S, T, H, deletion,
Position 186 to G, A, V, L, W, M, F, N, Q, Y, S, T, D,
E, R, H, deletion,
Position 194 to W, N, Q, Y, D, H,
Position 195 to W, P, Y, deletion,
10 Position 197 to G, V, L, I, W, P, M, F, Q, Y, S, T, H,
insertion, deletion,
Position 242 to insertion,
Position 249 to L, W, P, F, S, D, E, H, insertion, dele-
tion,
15 Position 252 to G, A, W, P, N, Q, Y, T, E, R, H, inser-
tion, deletion,
Position 254 to G, V, L, I, W, M, F, N, Q, Y, S, D, E,
R, H, insertion, deletion,
Position 257 to G, A, L, I, W, C, M, F, N, Q, Y, S, T,
20 D, E, K, H, insertion, deletion,
Position 260 to L, I, W, M, F, Y, T, H, insertion, dele-
tion,
Position 265 to V, L, I, W, M, F, Y, insertion, dele-
tion,

25

with the proviso that the amino acids of the parent enzyme are substituted to another amino acid.

90. The protein variant according to claims 76-81 having modi-
30 fied immunogenicity as compared to its parent protein having at least 81% homology to SEQ ID NO 10 comprising one or more of the following substitutions corresponding to any of the following in SEQ ID NO 10:

436

Position 4 to V, L, W, M, F, Y, R,
 Position 38 to V, L, I, W, M, F, N, Q, Y, T, H,
 Position 40 to V, L, I, W, M, F, N, Q, Y, T, R, H,
 Position 43 to G, L, H,
 5 Position 47 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
 T, D, E, R, H, insertion, deletion,
 Position 49 to G, A, V, I, W, P, M, F, N, Q, Y, S, T,
 D, E, R, H, insertion, deletion,
 Position 54 to V, L, I, W, M, F, S, R, deletion, inser-
 10 tion,
 Position 96 to L, W, F, Y, R, K, deletion,
 Position 99 to V, L, I, W, M, F, Q, Y, H, deletion,
 Position 113 to W, insertion,
 Position 131 to W, Y, R, deletion,
 15 Position 133 to A, L, I, W, M, F, Y, R,
 Position 137 to G, A, V, I, W, P, M, N, Y, H, insertion,
 deletion,
 Position 141 to G, V, L, I, W, P, M, F, Q, S, D, E, H,
 insertion, deletion,
 20 Position 144 to L, W, P, M, F, N, Q, Y, S, D, E, R, H,
 insertion, deletion,
 Position 170 to A, C, D, E, F, G, H, I, K, L, M, N, P,
 Q, R, S, T, V, W, Y;
 Position 173 to G, A, V, L, I, W, M, F, N, Q, Y, S, T,
 25 E, H, deletion,
 Position 181 to G, A, V, L, I, W, C, M, F, Q, Y, T, D,
 R, K, H, deletion,
 Position 185 to G, A, V, L, I, W, C, M, F, N, Q, Y, T,
 E, H, deletion,
 30 Position 186 to G, A, V, L, W, M, F, N, Q, Y, S, T, D,
 E, R, H, deletion,
 Position 188 to G, A, V, L, W, F, S, R, K, deletion,
 Position 194 to W, N, Q, Y, D, H,
 Position , 203 to V, F, Y, R, H,

437

Position 210 to W, F, Y,
 Position 211 to L, W, M, F, Y, H,
 Position 257 to G, A, L, I, W, C, M, F, N, Q, Y, S, T,
 D, E, K, H, insertion, deletion,
 5 Position 261 to L, N, S, H, insertion, deletion,
 Position 262 to G, A, V, L, I, W, P, F, N, Q, Y, T, D,
 E, R, H, insertion, deletion,
 Position 265 to V, L, I, W, M, F, Y, insertion, dele-
 tion,

10

with the proviso that the amino acids of the parent enzyme are substituted to another amino acid.

91. The protein variant according to claims 76-81 having modi-
 15 fied immunogenicity as compared to its parent protein having at least 81% homology to SEQ ID NO 11 comprising one or more of the following substitutions corresponding to any of the following in SEQ ID NO 11:

20 Position 38 to V, L, I, W, M, F, N, Q, Y, T, H,
 Position 40 to V, L, I, W, M, F, N, Q, Y, T, R, H,
 Position 45 to G, V, L, I, W, P, M, F, N, Q, Y, S, T,
 D, E, R, H,
 Position 47 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
 25 T, D, E, R, H, insertion, deletion,
 Position 49 to G, A, V, I, W, P, M, F, N, Q, Y, S, T,
 D, E, R, H, insertion, deletion,
 Position 50 to G, A, W, M, N, Q, Y, S, T, D, E, H, in-
 sertion, deletion,
 30 Position 52 to V, L, I, W, M, F, Y, S, T, R, deletion,
 insertion,
 Position 53 to A, V, L, I, W, M, F, N, Q, Y, S, D, E,
 H, deletion, insertion,
 Position 56 to G, V, L, I, W, M, F, N, Q, Y, S, T, H,

438

Position 58 to L, W, M, F, N, Y, R, insertion, deletion,
Position 96 to L, W, F, Y, R, K, deletion,
Position 97 to V, L, W, C, M, F, Y, H,
5 Position 98 to A, deletion,
Position 105 to insertion,
Position 109 to N, insertion,
Position 113 to W, insertion,
Position 115 to I, insertion,
10 Position 133 to A, L, I, W, M, F, Y, R,
Position 136 to G, A, W, P, N, Y, S, T, D, E, H, insertion,
deletion,
Position 137 to G, A, V, I, W, P, M, N, Y, H, insertion,
deletion,
15 Position 141 to G, V, L, I, W, P, M, F, Q, S, D, E, H,
insertion, deletion,
Position 158 to V, L, I, W, M, F, Y,
Position 159 to A, W, M, Y, T, R, H,
Position 172 to G, A, V, L, I, S, T, H, deletion,
20 Position 186 to G, A, V, L, W, M, F, N, Q, Y, S, T, D,
E, R, H, deletion,
Position 189 to W, F, deletion,
Position 192 to G, L, I, W, M, N, Q, Y, S, T, D, R, H,
deletion,
25 Position 195 to W, P, Y, deletion,
Position 197 to G, V, L, I, W, P, M, F, Q, Y, S, T, H,
insertion, deletion,
Position 257 to G, A, L, I, W, C, M, F, N, Q, Y, S, T,
D, E, K, H, insertion, deletion,
30 Position 261 to L, N, S, H, insertion, deletion,
Position 265 to V, L, I, W, M, F, Y, insertion, deletion,
deletion,

with the proviso that the amino acids of the parent enzyme are substituted to another amino acid.

5 92. The protein variant according to claims 76-81 having modified immunogenicity as compared to its parent protein having at least 81% homology to SEQ ID NO 33 comprising one or more of the following substitutions corresponding to any of the following in SEQ ID NO 33:

10

Position -6 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, Y, insertion, deletion,

Position -5 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W, Y, insertion, deletion,

15 Position -4 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y, insertion, deletion,

Position -2 to A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y, insertion, deletion,

Position -1 to G, V, L, I, W, C, M, F, N, Q, Y, S, T, D, E, R, H, deletion,

Position 1 to V, L, I, W, M, F, Y, S, T, R,

Position 2 to G, V, I, M, F, N, Q, Y, S, T, H,

Position 3a to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y, insertion, deletion,

25 Position 5 to V, L, I, W, M, F, N, Q, Y, T, R, H,

Position 6 to G, V, L, I, W, P, M, N, Q, T, D, E, R, H,

Position 7 to G, A, V, L, I, W, P, M, F, N, Q, Y, S, T, D, E, R, H,

30 Position 8 to G, A, L, W, P, C, M, F, N, Q, Y, S, T, D, E, R, K, H,

Position 10 to G, A, V, I, W, P, M, N, Q, Y, S, T, D, E, R, insertion, deletion,

440

- Position 12 to G, A, V, L, I, W, M, F, N, Q, Y, S, T,
D, E, insertion, deletion,
- Position 13 to G, L, I, W, P, M, F, N, Q, Y, S, D, E,
H, insertion, deletion,
- 5 Position 14 to V, L, I, W, P, M, F, N, Q, Y, T, R, H,
insertion, deletion,
- Position 15 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
T, E, H, insertion, deletion,
- Position 16 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
10 D, E, R, H, insertion, deletion,
- Position 17 to G, A, V, I, W, P, M, F, Y, H, insertion,
deletion,
- Position 18 to G, A, L, I, W, P, M, F, N, Q, Y, T, D,
E, H, insertion, deletion,
- 15 Position 19 to A, V, I, W, M, F, N, Y, S, T, D, R, H,
insertion, deletion,
- Position 21 to G, V, I, W, N, Q, Y, S, T, D, E, R, H,
insertion, deletion,
- Position 22 to G, V, L, I, W, M, F, Y, S, T, insertion,
20 deletion,
- Position 23 to G, A, V, L, I, W, M, F, Y, E, R, H, in-
sertion, deletion,
- Position 24 to G, V, L, I, W, M, F, N, Q, Y, S, D, E,
R, insertion, deletion,
- 25 Position 25 to G, A, V, L, I, W, M, F, N, Q, Y, S, T,
D, E, R, H, insertion, deletion,
- Position 26 to G, A, V, L, I, W, M, F, N, Q, Y, S, T,
D, E, R, H, insertion, deletion,
- Position 27 to G, L, I, W, P, M, F, Y, T, H,
- 30 Position 28 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
T, D, E, R, K, H, insertion, deletion,
- Position 28a to A, C, D, E, F, G, H, I, K, L, M, N, P,
Q, R, S, T, V, W, Y, insertion, deletion,

441

Position 29 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
 T, D, E, R, K, H, insertion, deletion,
 Position 33 to V, L, I, W, C, M, F, N, Q, Y, R, H,
 Position 35 to G, A, V, L, I, W, M, F, N, Q, Y, S, T,
 5 D, E, R, K, H, deletion,
 Position 37 to L, I, W, M, F, N, Q, Y, S, R, H,
 Position 40 to V, L, I, W, M, F, N, Q, Y, T, R, H,
 Position 42 to G, A, L, W, C, M, F, N, Q, Y, S, T, D,
 E, R, H,
 10 Position 43 to G, L, H,
 Position 44 to G, V, L, I, W, P, M, F, Y, S, T,
 Position 44a to A, C, D, E, F, G, H, I, K, L, M, N, P,
 Q, R, S, T, V, W, Y, insertion, deletion,
 Position 44b to A, C, D, E, F, G, H, I, K, L, M, N, P,
 15 Q, R, S, T, V, W, Y, insertion, deletion,
 Position 46 to G, A, L, I, W, P, M, F, Y, H, insertion,
 deletion,
 Position 48 to A, L, I, P, M, F, N, Y, D, H, insertion,
 deletion,
 20 Position 51 to V, L, I, W, M, F, N, Y, R, deletion, in-
 sertion,
 Position 52 to V, L, I, W, M, F, Y, S, T, R, deletion,
 insertion,
 Position 53 to A, V, L, I, W, M, F, N, Q, Y, S, D, E,
 25 H, deletion, insertion,
 Position 55 to G, A, V, L, I, W, C, M, F, N, Q, Y, T,
 D, E, R, K, H, deletion, insertion,
 Position 56 to G, V, L, I, W, M, F, N, Q, Y, S, T, H,
 Position 57 to G, A, V, L, I, W, M, F, N, Q, Y, S, T,
 30 D, E, R, K, H,
 Position 58 to L, W, M, F, N, Y, R, insertion, dele-
 tion,
 Position 61 to V, L, I, W, M, F, Y, insertion, dele-
 tion,

442

Position 64 to G, V, L, I, W, P, C, M, F, N, Q, Y, S,
 T, D, E, R, K, H, insertion, deletion,
 Position 75 to L,
 Position 81 to insertion,
 5 Position 86 to G, A, V, L, I, W, M, F, N, Q, Y, T, D,
 E, R, H,
 Position 87 to A, V, L, I, W, M, F, Q, Y, S, T, D, E,
 H,
 Position 88 to A, V, L, I, W, M, F, N, Q, Y, S, T, D,
 10 E, R, H,
 Position 89 to G, V, L, I, W, P, F, N, Y, T, E,
 Position 91 to G, A, V, L, I, W, P, M, N, Y, S, T, D,
 E, R, H, insertion, deletion,
 Position 92 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
 15 T, D, E, R, K, H, insertion, deletion,
 Position 94 to G, V, L, I, W, P, M, F, N, Y, T, D, E,
 K, H, insertion, deletion,
 Position 96 to L, W, F, Y, R, K, deletion,
 Position 97 to V, L, W, C, M, F, Y, H,
 20 Position 98 to deletion,
 Position 101 to V, I, W, M, F, N, Q, Y, H,
 Position 102 to V, L, I, W, M, F, Y, R, H, G, deletion,
 Position 108 to I, insertion,
 Position 109 to N, insertion,
 25 Position 111 to insertion,
 Position 112 to E, insertion,
 Position 113 to W, insertion,
 Position 114 to insertion,
 Position 115 to I, insertion,
 30 Position 117 to N, insertion,
 Position 118 to N, insertion,
 Position 119 to M, insertion,
 Position 127 to G, A, V, I, W, M, F, Y, R, H, L,
 Position 133 to A, L, I, W, M, F, Y, R,

443

Position 134 to L, I, W, F, N, Q, Y, R, H, insertion,
deletion,
Position 135 to G, L, P, C, N, Q, T, R, H,
Position 136 to G, A, W, P, N, Y, S, T, D, E, H, inser-
5 tion, deletion,
Position 137 to G, A, V, I, W, P, M, N, Y, H, insertion,
deletion,
Position 138 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
T, D, E, R, H, insertion, deletion,
10 Position 139 to G, A, V, L, I, W, P, C, M, F, N, Q, Y,
S, T, D, E, R, K, H, insertion, deletion,
Position 141 to G, V, L, I, W, P, M, F, Q, S, D, E, H,
insertion, deletion,
Position 142 to G, A, L, I, W, P, C, M, F, N, Q, Y, S,
15 T, D, E, R, K, H, insertion, deletion,
Position 144 to L, W, P, M, F, N, Q, Y, S, D, E, R, H,
insertion, deletion,
Position 145 to G, V, L, I, W, M, F, Q, Y, D, E, R, H,
insertion, deletion,
20 Position 146 to G, A, W, L, I, W, M, F, N, Q, Y, T, D,
E, R, H, insertion, deletion,
Position 147 to G, A, V, L, W, M, F, N, Q, Y, S, T, D,
E, R, K, H, insertion, deletion,
Position 148 to G, A, V, L, I, W, P, C, M, F, N, Q, Y,
25 S, T, D, E, R, K, H, insertion, deletion,
Position 156 to V, I, W, F, R,
Position 158 to V, L, I, W, M, F, Y,
Position 160 to W, M, F, Y, R, H,
Position 161 to I, W, M, F, Y, H,
30 Position 162 to I, W, F, Y, R,
Position 163 to V, W, M, F, H,
Position 167 to R, K,
Position 169 to C, E, F, G, H, I, K, L, M, N, Q, R, T,
V, W, Y,

444

Position 170 to A, C, D, E, F, G, H, I, K, L, M, N, P,
Q, R, S, T, V, W, Y, insertion, deletion,
Position 171 to D, deletion,
Position 174 to G, A, L, I, W, P, C, M, F, N, Q, Y, S,
5 T, D, E, R, K, H, insertion, deletion,
Position 176 to G, A, V, L, I, W, P, C, M, F, N, Q, Y,
S, T, D, E, R, K, H, insertion, deletion,
Position 182 to A, V, L, I, W, C, M, F, N, Q, Y, S, T,
D, E, H, deletion,
10 Position 186 to G, A, V, L, W, M, F, N, Q, Y, S, T, D,
E, R, H, deletion,
Position 188 to G, A, V, L, W, F, S, R, K, deletion,
Position 191 to A, V, L, I, W, M, F, Y, T, R, H, dele-
tion,
15 Position 192 to G, L, I, W, M, N, Q, Y, S, T, D, R, H,
deletion,
Position 193 to G, V, L, I, W, M, F, N, Q, Y, S, T, D,
E, R, H, deletion,
Position 194 to W, N, Q, Y, D, H,
20 Position 195 to W, P, Y, deletion,
Position 196 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
T, D, E, R, H, insertion, deletion,
Position 197 to G, V, L, I, W, P, M, F, Q, Y, S, T, H,
insertion, deletion,
25 Position 198 to G, A, L, I, W, P, C, M, F, N, Q, Y, S,
T, D, E, R, K, H, insertion, deletion,
Position 203 to V, F, Y, R, H,
Position 205 to W, F, Y, R, K,
Position 215 to A, L, I, W, M, F, Y,
30 Position 216 to A, L, I, W, M, F, Y, R,
Position 217 to W, R,
Position 219 to G, A, V, L, I, W, F, Y, R, H,
Position 233 to insertion,
Position 234 to I, insertion,

445

Position 236 to insertion,
 Position 237 to insertion,
 Position 238 to insertion,
 Position 239 to insertion,
 5 Position 240 to insertion,
 Position 243 to insertion,
 Position 246 to insertion,
 Position 247 to G, V, I, W, P, F, Y, S, T, R, insertion,
 deletion,
 10 Position 249 to L, W, P, F, S, D, E, H, insertion, dele-
 tion,
 Position 252 to G, A, W, P, N, Q, Y, T, E, R, H, inser-
 tion, deletion,
 Position 254 to G, V, L, I, W, M, F, N, Q, Y, S, D, E,
 15 R, H, insertion, deletion,
 Position 262 to G, A, V, L, I, W, P, F, N, Q, Y, T, D,
 E, R, H, insertion, deletion,
 Position 264a to A, C, D, E, F, G, H, I, K, L, M, N, P,
 Q, R, S, T, V, W, Y, insertion, deletion,
 20 Position 270 to G, L, I, W, P, M, F, N, Q, Y, S, T, D,
 E, R, K, H, insertion, deletion,
 Position 273 to G, A, V, L, I, W, P, M, F, N, Q, Y, S,
 T, D, E, R, K, H, insertion, deletion,
 Position 274 to W, P, M, F, N, Q, Y, T, D, E, R, H,
 25 Position 275 to G, A, V, L, I, W, M, F, N, Y, T, D, in-
 sersion, deletion,
 Position 276 to A, C, D, E, F, G, H, I, K, L, M, N, P,
 Q, R, S, T, V, W, Y, insertion, deletion,

30 with the proviso that the amino acids of the parent enzyme are
 substituted to another amino acid.

93. The protein variant according to claims 76-81 having modi-
 fied immunogenicity as compared to its parent protein having at

least 81% homology to SEQ ID NO 33 comprising one or more of the following substitutions corresponding to any of the following in SEQ ID NO 33:

- | | | | | |
|----|----------|-----|----|----------------------------------------------------------------------------------|
| 5 | Position | 5 | to | V, L, I, W, M, F, N, Q, Y, T, R, H, |
| | Position | 22 | to | G, V, L, I, W, M, F, Y, S, T, insertion,
deletion, |
| | Position | 26 | to | G, A, V, L, I, W, M, F, N, Q, Y, S, T,
D, E, R, H, insertion, deletion, |
| 10 | Position | 28 | to | G, A, V, L, I, W, P, M, F, N, Q, Y, S,
T, D, E, R, K, H, insertion, deletion, |
| | Position | 37 | to | L, I, W, M, F, N, Q, Y, S, R, H, |
| | Position | 40 | to | V, L, I, W, M, F, N, Q, Y, T, R, H, |
| | Position | 44 | to | G, V, L, I, W, P, M, F, Y, S, T, |
| 15 | Position | 51 | to | V, L, I, W, M, F, N, Y, R, deletion, in-
sertion, |
| | Position | 52 | to | V, L, I, W, M, F, Y, S, T, R, deletion,
insertion, |
| | Position | 55 | to | G, A, V, L, I, W, C, M, F, N, Q, Y, T,
D, E, R, K, H, deletion, insertion, |
| 20 | Position | 58 | to | L, W, M, F, N, Y, R, insertion, dele-
tion, |
| | Position | 61 | to | V, L, I, W, M, F, Y, insertion, dele-
tion, |
| 25 | Position | 64 | to | G, V, L, I, W, P, C, M, F, N, Q, Y, S,
T, D, E, R, K, H, insertion, deletion, |
| | Position | 87 | to | A, V, L, I, W, M, F, Q, Y, S, T, D, E,
H, |
| | Position | 97 | to | V, L, W, C, M, F, Y, H, |
| 30 | Position | 98 | to | deletion, |
| | Position | 101 | to | V, I, W, M, F, N, Q, Y, H, |
| | Position | 102 | to | V, L, I, W, M, F, Y, R, H, G, deletion, |
| | Position | 109 | to | N, insertion, |
| | Position | 112 | to | E, insertion, |

447

Position 118 to N, insertion,
 Position 127 to G, A, V, I, W, M, F, Y, R, H, L,
 Position 137 to G, A, V, I, W, P, M, N, Y, H, insertion,
 deletion,
 5 Position 146 to G, A, W, L, I, W, M, F, N, Q, Y, T, D,
 E, R, H, insertion, deletion,
 Position 156 to V, I, W, F, R,
 Position 158 to V, L, I, W, M, F, Y,
 Position 161 to I, W, M, F, Y, H,
 10 Position 188 to G, A, V, L, W, F, S, R, K, deletion,
 Position 192 to G, L, I, W, M, N, Q, Y, S, T, D, R, H,
 deletion,
 Position 194 to W, N, Q, Y, D, H,
 Position 195 to W, P, Y, deletion,
 15 Position 203 to V, F, Y, R, H,
 Position 216 to A, L, I, W, M, F, Y, R,
 Position 236 to insertion,
 Position 237 to insertion,
 Position 262 to G, A, V, L, I, W, P, F, N, Q, Y, T, D,
 20 E, R, H, insertion, deletion,
 Position 264a to A, C, D, E, F, G, H, I, K, L, M, N, P,
 Q, R, S, T, V, W, Y, insertion, deletion,

with the proviso that the amino acids of the parent enzyme are
 25 substituted to another amino acid.

94. The protein variant according to claim 76, wherein the
 lipolytic enzyme comprises one or more of the following substi-
 30 tutions corresponding to any of the following in SEQ ID NO: 1:

Q15 to A, C, D, E, F, G, I, K, L, M, N, P, R, S, T, V, W, Y;
 Y16 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W;
 A18 to C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;

- A19 to C, D, E, F, G, H, I, K, L, M, N, Q, R, S, V, W, Y;
A20 to C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y;
N25 to A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, Y;
N26 to A, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, Y;
5 E43 to A, C, D, F, G, H, I, K, L, M, N, R, S, T, V, W, Y;
V44 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, W, Y;
K46 to A, C, D, E, F, G, H, I, L, M, N, Q, S, T, V, W, Y;
A47 to C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, Y;
A49 to C, D, E, F, G, H, I, K, L, M, N, Q, R, S, V, W, Y;
10 L52 to A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, Y;
Y53 to A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W;
S54 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, T, V, W, Y;
G65 to A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
L67 to A, C, D, E, F, G, H, I, K, M, N, Q, R, S, T, V, W, Y;
15 A68 to C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
L69 to A, C, D, E, F, G, H, I, K, M, N, P, Q, S, T, V, W, Y;
T72 to A, C, D, E, F, G, H, I, L, M, N, P, Q, R, S, V, W, Y;
K74 to A, C, D, E, F, G, H, I, L, M, N, P, Q, R, S, T, V, W, Y;
L75 to A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, Y;
20 V77 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, Y;
S79 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W, Y;
R81 to A, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, Y;
S83 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, V, W, Y;
S85 to A, D, E, G, H, I, L, M, N, Q, V, W, Y;
25 W89 to A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, Y;
L97 to A, C, D, E, F, G, H, I, K, N, P, R, S, T, W, Y;
K98 to A, C, G, H, L, M, N, P, Q, S, T, V, W, Y;
E99 to C, F, G, I, M, P, W, Y;
G106 to A, C, D, E, F, H, I, K, L, M, N, P, Q, R, T, V, W, Y;
30 C107 to A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
R108 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, Y;
G109 to A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, W, Y;
T123 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W, Y;
L124 to A, C, D, E, F, G, H, I, K, M, N, P, Q, R, T, V, W, Y;

- K127 to A, D, E, F, G, H, I, L, M, N, P, Q, R, S, T, V, W, Y;
E129 to A, C, D, F, G, H, I, L, M, N, P, Q, R, S, T, V, W, Y;
A131 to C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
V132 to A, C, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, W, Y;
5 Y138 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W;
V140 to A, C, D, E, F, G, H, I, K, L, M, N, P, R, S, T, W, Y;
L147 to A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, Y;
A150 to C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
T153 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W, Y;
10 Y164 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W;
D165 to A, C, E, F, G, H, I, K, L, M, N, Q, S, T, V, W, Y;
D167 to A, C, E, F, H, I, L, M, N, P, Q, S, T, V, W, Y;
S170 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, T, V, W, Y;
Y171 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W;
15 G172 to A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
A173 to C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y;
P174 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y;
R175 to A, C, D, E, F, G, H, I, K, L, M, N, Q, S, T, V, W, Y;
V176 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, Y;
20 G177 to A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
R179 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, Y;
A182 to C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
Y194 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W;
H198 to A, C, D, E, F, G, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
25 N200 to A, C, D, E, F, G, H, I, K, L, M, P, Q, S, T, V, W, Y;
P207 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y;
P208 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y;
R209 to C, D, F, G, H, I, K, L, M, N, Q, T, V, W, Y;
G212 to A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
30 S214 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W, Y;
H215 to A, C, D, E, F, G, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
S216 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, T, V, W, Y;
S217 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W, Y;
P218 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y;

450

E219 to C, D, F, H, I, M, P, W, Y;
Y220 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W;
K223 to A, C, D, E, F, G, H, I, L, M, N, Q, S, T, V, W, Y;
S224 to A, C, D, E, F, G, H, I, K, L, M, N, Q, T, V, W, Y;
5 D234 to C, E, F, H, I, M, W;
I235 to A, C, D, E, F, G, H, K, L, M, N, P, Q, R, S, T, V, W, Y;
K237 to A, C, D, E, F, G, H, I, L, N, P, Q, S, T, V, W, Y;
I238 to A, C, D, E, F, G, H, K, L, M, N, P, Q, R, S, T, V, W, Y;
D242 to C, E, F, G, H, I, M, P, W, Y;
10 A243 to C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W, Y;
P250 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y;
P253 to A, C, D, E, F, G, H, I, K, L, M, N, Q, S, T, V, W, Y;
D254 to C, E, F, H, I, M, P, Y;
I255 to C, D, E, F, H, L, M, N, Q, W, Y;
15 P256 to C, E, F, G, H, I, K, L, M, N, Q, R, V, W, Y;
Y261 to A, C, E, F, G, H, L, M, N, P, Q, R, S, T, V.

95. The protein variant according to claim 94, wherein the
20 lipolytic enzyme comprises one or more of the substitutions corresponding to any of the following in SEQ ID NO: 1:

G65 to A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
L67 to A, C, D, E, F, G, H, I, K, M, N, Q, R, S, T, V, W, Y;
25 R81 to A, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, Y;
S83 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, V, W, Y;
S85 to A, D, E, G, H, I, L, M, N, Q, V, W, Y;
L97 to A, C, D, E, F, G, H, I, K, N, P, R, S, T, W, Y;
L124 to A, C, D, E, F, G, H, I, K, M, N, P, Q, R, T, V, W, Y;
30 E129 to A, C, D, F, G, H, I, L, M, N, P, Q, R, S, T, V, W, Y;
Y164 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W;
R179 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, Y;
A182 to C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
P207 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y;

451

P208 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y;
 R209 to C, D, F, G, H, I, K, L, M, N, Q, T, V, W, Y;
 G212 to A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
 S214 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W, Y;
 5 H215 to A, C, D, E, F, G, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
 S216 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, T, V, W, Y;
 S217 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W, Y;
 P218 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y;
 E219 to C, D, F, H, I, M, P, W, Y;
 10 Y220 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W;
 A243 to C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W, Y;
 P250 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y;
 P253 to A, C, D, E, F, G, H, I, K, L, M, N, Q, S, T, V, W, Y;

15

96. The protein variant according to claim 95, wherein the lipolytic comprises one or more of the following substitutions:

P207 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y;
 20 P208 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y;
 S214 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W, Y;
 S216 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, T, V, W, Y;
 S217 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W, Y;
 A243 to C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W, Y;
 25 P250 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y;
 P253 to A, C, D, E, F, G, H, I, K, L, M, N, Q, S, T, V, W, Y;

97. The protein variant according to claim 94-96, wherein the
 30 parent lipolytic enzyme has at least 80% homology with SEQ ID NO.1.

98. The protein variant according to claim 76, wherein the carbohydrase is a glucoamylase comprising one or more of the fol-

452

lowing substitutions corresponding to any of the following in
SEQ ID NO 36:

Position 68 to A, C, D, E, F, G, H, I, K, L, M, N, P,
5 Q, R, S, T, V, W, Y, deletion, insertion,
Position 94 to insertion,
Position 102 to insertion,
Position 122 to insertion,
Position 125 to insertion,
10 Position 272 to A, C, D, E, F, G, H, I, K, L, M, N, P,
Q, R, S, T, V, W, Y, deletion, insertion,
Position 345 to A, C, D, E, F, G, H, I, K, L, M, N, Q,
R, S, T, V, W, Y, deletion, insertion,
Position 348 to A, C, D, E, F, G, H, I, K, L, M, N, P,
15 Q, R, S, T, V, W, Y, deletion, insertion,
Position 353 to insertion,
Position 357 to insertion,
Position 359 to insertion,
Position 450 to insertion,
20 Position 451 to insertion,
Position 468 to insertion,

with the proviso that the amino acids of the parent enzyme are
substituted to another amino acid.

25

99. The protein variant according to claims 98, wherein the en-
zyme is at least 81 % homologous, preferably 90% homologous,
more preferably 95% homologous, most preferably 99% homologous
to Carezyme core (SEQ ID NO 36).

30

100. The protein variant according to claim 76, wherein the car-
bohydrase is a Thermamyl-like α -amylase comprising one or more
of the following substitutions corresponding to any of the fol-
lowing in SEQ ID NO 2:

Position TYR 8 to A, C, D, G, K, M, P, R, W,
Y, insertion;

Position ASP 25 to A, C, D, E, F, G, H, I,
5 K, L, M, N, P, Q, R, S, T, V, W, Y, insertion;

Position ASP 26 to A, C, D, E, F, G, H, I,
K, L, M, P, Q, R, S, T, V, W, Y;

Position ALA 27 to C, D, E, F, G, H, I, K,
L, M, N, P, Q, R, S, T, V, W, Y;

10 Position SER 28 to A, C, D, F, G, H, I, K,
L, M, P, Q, R, S, T, V, W, Y;

Position ASN 29 to A, C, D, G, K, M, P, R,
W, Y;

Position ARG 31 to A, C, D, E, F, G, H, I,
15 K, L, M, N, P, Q, R, T, V, W, Y;

Position PRO 41 to C, D, E, F, G, H, I, K,
L, M, N, P, Q, R, S, T, V, W, Y, deletion, insertion;

Position PRO 42 to C, D, E, F, G, H, I, K,
L, M, N, P, Q, R, S, T, V, W, Y, deletion, insertion;

20 Position TYR 54 to A, C, D, E, G, K, M, P,
R, Y, insertion;

Position TYR 57 to A, C, D, G, K, M, P, R,
W, Y, insertion;

Position LEU 62 to A, C, D, E, F, G, H, I,
25 K, L, M, N, P, Q, R, S, T, V, W, Y, deletion, insertion;

Position GLY 63 to A, C, D, E, F, G, H, I,
K, L, M, N, P, Q, R, S, T, V, W, Y, deletion, insertion;

Position GLY 76 to A, C, D, E, F, G, H, I,
K, L, M, N, P, Q, R, S, T, V, W, Y, deletion, insertion;

30 Position ARG 78 to A, C, D, E, F, G, H, I,
K, L, M, N, P, Q, R, S, T, V, W, Y, deletion, insertion;

Position SER 79 to A, C, D, E, F, G, H, I,
K, L, M, N, P, Q, R, S, T, V, W, Y, deletion, insertion;

454

Position LEU 88 to A, C, D, E, F, G, H, I,
K, L, M, N, P, Q, R, S, T, V, W, Y, deletion, insertion;
Position GLY 92 to A, C, D, E, F, G, H, I,
K, L, M, N, P, Q, R, S, T, V, W, Y, deletion, insertion;
5 Position ASN 102 to A, C, E, F, G, H, I, L,
M, P, Q, S, T, V, W, Y, insertion;
Position ALA 107 to A, C, D, E, F, G, H, I,
K, L, M, N, P, Q, R, S, T, V, W, Y, deletion, insertion;
Position ASP 108 to A, C, D, E, F, G, H, I,
10 K, L, M, N, P, Q, R, S, T, V, W, Y, deletion, insertion;
Position ALA 109 to A, C, D, E, F, G, H, K,
M, N, P, Q, R, S, W, Y;
Position LYS 138 to A, C, E, F, G, I, L, M,
N, P, Q, S, T, V, W, insertion;
15 Position ASP 140 to A, C, E, F, G, I, K, L,
M, N, P, Q, S, T, V, W;
Position PRO 142 to C, D, E, F, G, H, I, K,
L, M, N, Q, R, S, T, V, W, Y;
Position ARG 144 to A, C, D, E, F, G, H, I,
20 K, L, M, N, P, Q, R, S, T, V, Y;
Position GLN 170 to A, C, E, F, G, H, I, K,
L, M, N, P, Q, R, S, T, V, W, Y;
Position ILE 173 to A, C, D, E, F, G, H, I,
K, L, M, N, P, Q, R, S, T, V, W, Y;
25 Position ASP 195 to A, C, D, E, F, G, H, I,
K, L, M, P, Q, R, S, T, V, W, Y, deletion, insertion;
Position TYR 196 to A, C, D, G, K, M, P, R,
W, Y, insertion;
Position ASP 232 to A, C, D, E, F, G, H, I,
30 K, L, M, N, P, Q, R, S, T, V, W, Y, deletion, insertion;
Position ALA 233 to A, C, D, E, I, K, L, M,
N, P, Q, R, W, Y, deletion, insertion;
Position GLN 331 to A, C, D, F, G, H, I, K,
M, N, P, Q, R, S, T, V, W, Y, deletion, insertion;

455

Position TYR 349 to A, C, D, G, K, M, P, R,
W, Y, insertion;

Position ILE 352 to A, C, D, E, F, G, H, I,
K, L, M, N, P, Q, R, S, T, V, W, Y, deletion, insertion;

5 Position GLN 357 to C, D, E, G, H, I, K, L,
M, N, P, Q, R, S, T, V, W, Y, deletion, insertion;

Position ASP 366 to A, C, D, E, F, G, H, I,
K, L, M, P, Q, R, S, T, V, W, Y, deletion, insertion;

Position TYR 367 to C, E, F, H, K, M, N, P,
10 Q, R, V, W, insertion;

Position TYR 368 to A, C, D, G, K, M, P, R,
W, Y, insertion;

Position ILE 370 to A, C, D, E, F, G, H, I,
K, L, M, N, P, Q, R, S, T, V, W, Y;

15 Position ALA 380 to A, C, D, E, F, G, H, I,
K, L, M, N, P, Q, R, T, V, W, Y;

Position LYS 381 to A, C, D, E, F, G, H, I,
K, L, M, N, P, Q, S, T, V, W, Y, deletion, insertion;

Position ILE 382 to A, C, D, E, F, G, H, I,
20 K, L, M, N, P, Q, R, S, T, V, W, Y, deletion, insertion;

Position PRO 384 to C, D, E, F, G, H, I, K,
M, N, P, Q, R, S, T, V, W, deletion, insertion;

Position LEU 386 to A, C, D, E, F, G, H, I,
25 K, L, M, N, P, Q, R, S, T, V, W, Y, deletion, insertion;

Position ARG 389 to A, C, D, E, F, G, H, I,
K, L, M, N, P, Q, R, S, T, V, W, Y, deletion, insertion;

Position GLN 390 to A, C, D, E, F, G, H, I,
L, M, N, P, Q, S, T, V, W, Y;

30

with the proviso that the amino acids of the parent enzyme are substituted to another amino acid.

456

101. The protein variant according to claim 100, wherein the amylase comprises one or more of the following substitutions:

- Position PRO 41 to C, D, E, F, G, H, I,
 5 K, L, M, N, P, Q, R, S, T, V, W, Y, deletion, insertion;
- Position PRO 42 to C, D, E, F, G, H, I,
 K, L, M, N, P, Q, R, S, T, V, W, Y, deletion, insertion;
- Position ALA 109 to A, C, D, E, F, G, H,
 K, M, N, P, Q, R, S, W, Y;
- 10 Position LYS 138 to A, C, E, F, G, I, L,
 M, N, P, Q, S, T, V, W;
- Position ASP 140 to A, C, E, F, G, I, K,
 L, M, N, P, Q, S, T, V, W;
- Position PRO 142 to C, D, E, F, G, H, I,
 15 K, L, M, N, Q, R, S, T, V, W, Y;
- Position ARG 144 to A, C, D, E, F, G, H,
 I, K, L, M, N, P, Q, R, S, T, V, Y;
- Position ASP 366 to A, C, D, E, F, G, H,
 I, K, L, M, P, Q, R, S, T, V, W, Y, deletion, insertion;
- 20 Position TYR 367 to C, E, F, H, K, M, N,
 P, Q, R, V, W, insertion;
- Position TYR 368 to A, C, D, G, K, M, P,
 R, W, Y, insertion;
- Position ALA 380 to A, C, D, E, F, G, H,
 25 I, K, L, M, N, P, Q, R, T, V, W, Y;
- Position LYS 381 to A, C, D, E, F, G, H,
 I, K, L, M, N, P, Q, S, T, V, W, Y, deletion, insertion;
- Position ILE 382 to A, C, D, E, F, G, H,
 I, K, L, M, N, P, Q, R, S, T, V, W, Y, deletion, insertion;
- 30 Position PRO 384 to C, D, E, F, G, H, I,
 K, M, N, P, Q, R, S, T, V, W, deletion, insertion;
- Position ARG 389 to A, C, D, E, F, G, H,
 I, K, L, M, N, P, Q, R, S, T, V, W, Y, deletion, insertion;

with the proviso that the amino acids of the parent enzyme are substituted to another amino acid.

5

102. The protein variant according to claims 100-101, wherein the enzyme is at least 81 % homologous, preferably 90% homologous, more preferably 95% homologous, most preferably 99% homologous to SEQ ID NO 2.

10

103. The protein variant according to claims 100-102, wherein the enzyme has any of the amino acid sequence of SEQ ID NO 2, 4, 5, 37.

15 104. A cellulase variant of a microbial parent cellulase having a catalytically active domain classified in family 45, said variant comprises a substitution of one or more amino acid residues at a position corresponding to a position in SEQ ID NO:4 from the group consisting of:

20

Position 1 to C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;

Position 2 to A, C, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, Y;

25 Position 7 to A, C, D, E, F, G, H, K, L, M, N, P, Q, S, T, V, Y;

Position 20 to C, D, F, H, I, L, M, N, P, Q, S, T, V, W, Y;

Position 23 to A, C, D, E, F, G, H, I, K, L, M, N, Q,
30 R, S, T, V, W, Y;

Position 27 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y;

Position 29 to A, C, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;

458

Position 36 to A, C, D, E, F, G, H, I, K, L, M, N, P,
 R, S, T, V, W, Y;
 Position 37 to C, D, E, F, G, H, I, K, L, M, P, Q, T,
 V, W, Y;
 5 Position 38 to A, C, D, E, G, H, K, M, N, P, R, S, T,
 V, W, Y;
 Position 40 to A, C, E, F, G, H, I, K, L, M, N, P, Q,
 R, S, T, V, W, Y;
 Position 41 to A, C, D, E, G, H, I, K, L, M, N, P, Q,
 10 R, S, T, V, W, Y;
 Position 44 to A, C, D, E, F, H, I, L, M, N, S, T, W,
 Y;
 Position 54 to A, C, D, E, G, H, I, K, L, M, N, P, Q,
 R, S, T, V, W;
 15 Position 59 to A, C, D, E, F, G, H, I, K, L, M, N, P,
 R, S, T, V, W, Y;
 Position 61 to A, C, D, E, F, G, H, I, K, L, M, N, Q,
 R, S, T, V, W, Y;
 Position 62 to A, C, D, G, H, I, K, L, M, N, P, Q, R,
 20 S, T, V, Y;
 Position 83 to C, D, E, F, G, H, I, K, L, M, N, P, Q,
 R, S, T, V, W, Y;
 Position 84 to A, C, D, E, F, H, I, K, L, M, N, P, Q,
 R, S, T, V, W, Y;
 25 Position 95 to A, C, D, F, G, H, I, K, L, M, N, P, Q,
 R, S, V, W, Y;
 Position 96 to A, C, D, E, F, G, H, I, K, L, M, N, P,
 Q, V, W, Y;
 Position 97 to C, D, E, F, H, I, K, L, M, N, P, Q, R, S, V, W,
 30 Y;
 Position 98 to C, D, E, F, G, H, I, K, L, M, N, Q, R,
 S, T, V, W, Y;
 Position 100 to C, D, E, F, G, H, I, K, L, M, N, P, Q,
 S, T, V, W, Y;

459

- Position 101 to A, C, D, E, F, H, I, K, L, M, N, P, Q,
R, S, T, V, W, Y;
- Position 131 to C, D, E, F, K, M, P, R, S, W, Y;
- Position 133 to A, C, E, F, G, H, I, L, M, P, R, S, T,
5 V, W, Y;
- Position 134 to C, D, E, F, H, I, K, L, M, N, P, Q, R,
S, T, V, W, Y;
- Position 136 to A, C, E, F, G, H, I, K, L, M, N, P, Q,
R, S, V, W, Y;
- 10 Position 142 to A, C, E, F, G, H, I, K, M, N, P, Q, R, V, W, Y;
- Position 143 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T,
V, W, Y;
- Position 145 to C, E, F, G, H, I, K, L, M, P, R, S, T, V, W, Y;
- Position 146 to A, C, D, F, G, H, I, K, L, M, N, P, T,
15 V, W, Y;
- Position 151 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T,
V, W, Y;
- Position 153 to C, D, E, F, G, H, I, M, N, P, Q, S, T,
V, W, Y;
- 20 Position 154 to A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T,
V, W, Y;
- Position 155 to A, C, D, F, G, H, I, K, L, M, N, P, Q, R, S, T,
V, W, Y;
- Position 157 to A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T,
25 V, W, Y;
- Position 158 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, S, T,
V, W, Y;
- Position 160 to A, C, D, E, F, G, H, I, K, L, M, N, Q,
R, S, T, V, W, Y;
- 30 Position 162 to C, D, E, F, G, H, I, K, L, M, N, Q, R,
S, T, V, W, Y;
- Position 163 to A, C, D, E, F, G, H, I, K, M, P, Q, R,
S, T, Y;

460

Position 164 to A, C, D, E, F, G, H, I, L, M, N, P, Q,
 R, S, T, V, W, Y;
 Position 165 to A, C, D, E, F, G, H, I, K, L, M, N, Q,
 R, S, T, V, W, Y;
 5 Position 168 to A, C, D, E, F, G, H, I, K, L, M, N, P,
 Q, R, S, T, V, W;
 Position 169 to A, C, D, E, G, H, I, K, L, M, N, P, Q,
 R, S, T, V, Y;
 Position 170 to A, C, D, E, G, H, I, K, L, M, N, P, Q,
 10 S, T, V, W, Y;
 Position 174 to A, C, D, E, G, H, I, K, L, N, P, Q, R,
 S, T, V, Y;
 Position 176 to A, C, E, F, G, H, I, K, L, M, P, Q, R,
 S, T, V, W, Y;
 15 Position 177 to C, D, E, F, G, H, I, K, L, M, P, Q, R,
 S, T, V, W, Y;
 Position 178 to A, C, E, F, G, H, I, K, L, M, Q, R, S,
 T, V, W, Y;
 Position 180 to A, C, D, E, F, G, H, I, K, M, N, Q, R,
 20 S, T, V, W, Y;
 Position 183 to A, C, D, E, F, G, H, I, K, L, M, N, P,
 Q, R, T, V, W, Y;
 Position 191 to C, D, E, F, G, H, I, K, L, M, N, P, Q,
 R, S, T, V, W, Y;
 25 Position 195 to C, D, E, F, G, H, I, K, L, M, N, P, Q,
 R, S, T, V, W, Y;
 Position 197 to A, C, D, E, F, G, H, I, K, L, M, N, P,
 Q, R, V, W, Y;
 and
 30 Position 200 to A, C, D, E, F, G, H, I, K, L, M, N, P,
 Q, S, T, V, W, Y;

105. The protein variant according to claim 104, wherein the carbohydrase comprises one or more of the following substitutions:

- 5 Position 20 to C, D, F, H, I, L, M, N, P, Q, S, T, V, W, Y;
- Position 23 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y;
- Position 27 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y;
- 10 Position 83 to C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
- Position 84 to A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
- 15 Position 95 to A, C, D, F, G, H, I, K, L, M, N, P, Q, R, S, V, W, Y;
- Position 96 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, V, W, Y;
- Position 97 to C, D, E, F, H, I, K, L, M, N, P, Q, R, S, V, W, Y;
- 20 Position 98 to C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y;
- Position ALA 100 to C, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, Y;
- 25 and
- Position 101 to A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;
- Position 131 to C, D, E, F, K, M, P, R, S, W, Y;
- Position 142 to A, C, E, F, G, H, I, K, M, N, P, Q, R, V, W, Y;
- 30 Position 143 to A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, Y;
- Position 145 to C, E, F, G, H, I, K, L, M, P, R, S, T, V, W, Y;
- Position 151 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W, Y;

462

Position 154 to A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, Y;

Position 155 to A, C, D, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;

5 Position 157 to A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;

Position 158 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, Y;

10

106. The protein variant according to claims 104-105, wherein the enzyme is at least 81 % homologous, preferably 90% homologous, more preferably 95% homologous, most preferably 99% homologous to Carezyme core (SEQ ID NO 4).

15

107. The protein variant according to claim 76, wherein the laccase is a Coprinus-like laccase.

20 108. The protein variant according to claim 107, wherein Laccase comprises one or more of the following substitutions corresponding to any of the following in SEQ ID NO 3:

Position 5 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;

Position 8 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;

Position 10 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;

30 Position 12 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;

Position 22 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;

463

Position 23 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 30 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

5 Position 39 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 40 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 41 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
10 T, V, W, Y;

Position 42 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 43 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

15 Position 51 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 53 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 55 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
20 T, V, W, Y;

Position 58 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 59 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

25 Position 60 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 71 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 72 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
30 T, V, W, Y;

Position 78 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 79 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

464

Position 80 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 100 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

5 Position 101 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 102 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 112 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
10 T, V, W, Y;

Position 113 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 114 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

15 Position 118 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 139 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 142 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
20 T, V, W, Y;

Position 155 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 157 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

25 Position 165 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 166 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 168 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
30 T, V, W, Y;

Position 175 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 180 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

465

Position 183 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 186 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

5 Position 190 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 191 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 192 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
10 T, V, W, Y;

Position 193 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 211 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

15 Position 213 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 231 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 234 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
20 T, V, W, Y;

Position 236 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 241 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

25 Position 251 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 257 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 259 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
30 T, V, W, Y;

Position 265 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 275 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

466

Position 286 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 294 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

5 Position 295 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 296 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 299 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
10 T, V, W, Y;

Position 300 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 301 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

15 Position 302 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 306 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 313 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
20 T, V, W, Y;

Position 314 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 315 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

25 Position 320 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 321 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 322 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
30 T, V, W, Y;

Position 324 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 329 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 332 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 335 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

5 Position 336 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 339 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 344 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
10 T, V, W, Y;

Position 345 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 348 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

15 Position 349 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 350 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 366 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
20 T, V, W, Y;

Position 367 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 369 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

25 Position 370 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 371 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 372 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
30 T, V, W, Y;

Position 375 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 378 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 379 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 389 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

5 Position 390 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 409 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 410 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
10 T, V, W, Y;

Position 414 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 416 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

15 Position 418 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 419 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 420 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
20 T, V, W, Y;

Position 430 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 432 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

25 Position 433 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 434 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 442 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
30 T, V, W, Y;

Position 443 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 445 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

469

Position 446 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 469 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

5 Position 473 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 485 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 488 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
10 T, V, W, Y;

Position 490 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 491 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

15 Position 492 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 493 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 494 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
20 T, V, W, Y;

Position 495 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 496 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

25 Position 499 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 500 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
T, V, W, Y;

Position 501 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S,
30 T, V, W, Y;

with the proviso that the amino acids of the parent protein is
substituted to another amino acid.

109. The protein variant according to claim 108, wherein Laccase comprises one or more of the following substitutions corresponding to any of the following in SEQ ID NO 3:

5

Position 59 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;

Position 96 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;

10 Position 100 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;

Position 181 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;

15 Position 348 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;

Position 369 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;

Position 414 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;

20 Position 432 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;

Position 493 to A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y;

25 with the proviso that the amino acids of the parent protein is substituted to another amino acid.

110. A subtilisin variant comprising one or more of the insertions, substitutions and/or deletions in any of the positions
30 according to claims 77-93.

111. The variant according to claim 110, wherein the subtilisin has at least 60%, preferably at least 70%, more preferably at least 80, even more preferably at least 90, still more prefera-

bly at least 95%, most preferably at least 99% homology to SEQ ID NO; 10.

112. A lipolytic enzyme comprising one or more of the insertions, substitutions and/or deletions in any of the positions according to claims 94-97.

113. A glycoamylase variant comprising one or more of the insertions, substitutions and/or deletions in any of the positions according to claims 98-99.

114. The variant according to claim 113, wherein the variant has at least 60%, preferably at least 70%, more preferably at least 80, even more preferably at least 90, still more preferably at least 95%, most preferably at least 99% homology to SEQ ID NO: 36.

115. A Thermamyl-like α -amylase comprising one or more of the insertions, substitutions and/or deletions in any of the positions according to claims 100-103.

116. The variant according to claim 115, wherein the variant has at least 60%, preferably at least 70%, more preferably at least 80, even more preferably at least 90, still more preferably at least 95%, most preferably at least 99% homology to SEQ ID NO; 2.

117. A cellulase variant comprising one or more of the insertions, substitutions and/or deletions in any of the positions according to claims 104-106.

118. The variant according to claim 117, wherein the variant has at least 60%, preferably at least 70%, more preferably at least 80, even more preferably at least 90, still more preferably at

472

least 95%, most preferably at least 99% homology to SEQ ID NO;
10.

119. A coprinus-like laccase variant comprising one or more of
5 the insertions, substitutions and/or deletions in any of the po-
sitions according to claims 107-109.

120. A composition comprising a protein variant as defined in
any of claims 22-119.

10

121. The composition according to claim 120, wherein the compo-
sition is in form of a pharmaceutical composition such as a vac-
cine.

15 122. The composition according to claim 120, wherein the compo-
sitions is in form of a industrial composition such as, a deter-
gent composition, personal care composition.

123. The use of the composition as defined in claim 120 for the
20 production of a pharmaceutical.

124. The use of the composition as defined in claim 120 for in-
dustrial application.

25 125. A DNA construct comprising a DNA sequence encoding a pro-
tein variant as defined in any of claims 22-119.

126. An expression vector comprising a DNA construct according
to claim 125.

30

127. A host cell which is capable of expressing a polypeptide
and comprising a DNA construct as defined in claim 125.

473

128. A host cell which is capable of expressing a polypeptide and which is transformed by an expression vector according to claim 126.

5 129. A host according to claims 127-128, which is a fungal cell, an insect cell, a mammalian cell, or a plant cell.

130. A method of producing a protein variant having reduced immunogenicity as compared to the parent protein, comprising:

10

- culturing a host according to any of claims 127-129 in a suitable culture medium to obtain expression and secretion of the protein into the medium, followed by

15 - isolation of the protein from the culture medium.

131. A kit for characterizing specificity of the allergic response of a patient, comprising a set of antibody binding peptide sequences corresponding to at least one epitope on at least one potential allergen.

132. The kit according to claim 131, for which the antibody binding sequences each are specific for one out of a known range of allergens, such that the characterization of allergic specificity becomes less susceptibility to cross-reactivity interferences.

30 133. A kit according to claims 131-132, which further comprises other diagnostic reagents, which facilitate determination of the serum response to each of the antibody binding sequences.

134. A kit according to claims 131-133, which further comprises

474

allergen vaccines, which can be administered to the patient according to the test results obtained using the antibody binding sequences.

1/1

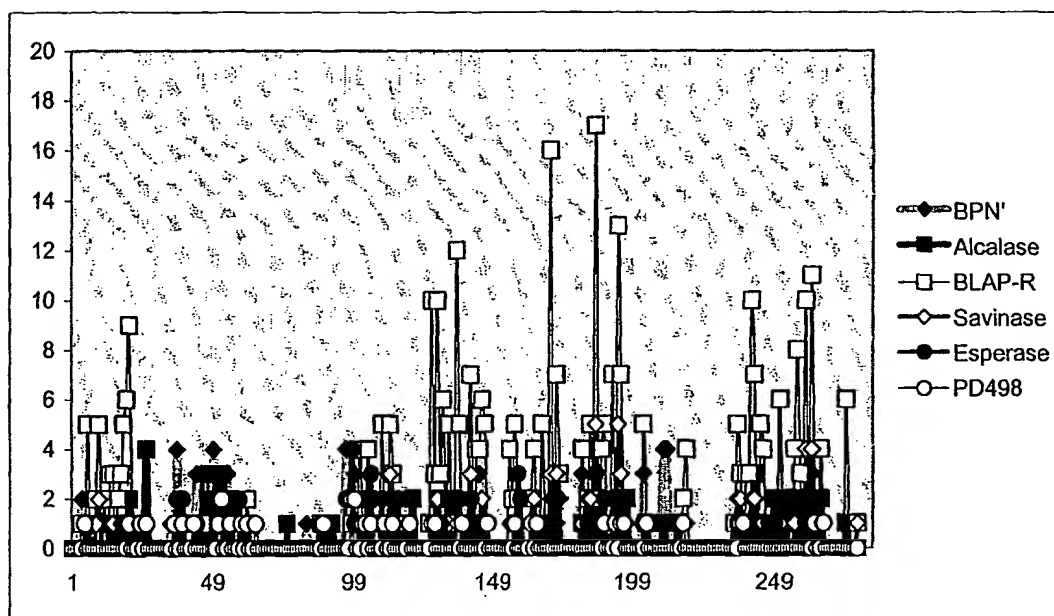


FIG. 1

SEQUENCE LISTING

<110> NOVOZYMES A/S

<120> PROTEIN VARIANTS HAVING MODIFIED IMMUNOGENICITY

<130> 10021

<160> 37

<170> PatentIn version 3.0

<210> 1

<211> 269

<212> PRT

<213> T. lanuginosus

<400> 1

Glu Val Ser Gln Asp Leu Phe Asn Gln Phe Asn Leu Phe Ala Gln Tyr
1 5 10 15
Ser Ala Ala Ala Tyr Cys Gly Lys Asn Asn Asp Ala Pro Ala Gly Thr
20 25 30
Asn Ile Thr Cys Thr Gly Asn Ala Cys Pro Glu Val Glu Lys Ala Asp
35 40 45
Ala Thr Phe Leu Tyr Ser Phe Glu Asp Ser Gly Val Gly Asp Val Thr
50 55 60
Gly Phe Leu Ala Leu Asp Asn Thr Asn Lys Leu Ile Val Leu Ser Phe
65 70 75 80
Arg Gly Ser Arg Ser Ile Glu Asn Trp Ile Gly Asn Leu Asn Phe Asp
85 90 95
Leu Lys Glu Ile Asn Asp Ile Cys Ser Gly Cys Arg Gly His Asp Gly
100 105 110
Phe Thr Ser Ser Trp Arg Ser Val Ala Asp Thr Leu Arg Gln Lys Val
115 120 125
Glu Asp Ala Val Arg Glu His Pro Asp Tyr Arg Val Val Phe Thr Gly

130 135 140
 His Ser Leu Gly Gly Ala Leu Ala Thr Val Ala Gly Ala Asp Leu Arg
 145 150 155 160
 Gly Asn Gly Tyr Asp Ile Asp Val Phe Ser Tyr Gly Ala Pro Arg Val
 165 170 175
 Gly Asn Arg Ala Phe Ala Glu Phe Leu Thr Val Gln Thr Gly Gly Thr
 180 185 190
 Leu Tyr Arg Ile Thr His Thr Asn Asp Ile Val Pro Arg Leu Pro Pro
 195 200 205
 Arg Glu Phe Gly Tyr Ser His Ser Ser Pro Glu Tyr Trp Ile Lys Ser
 210 215 220
 Gly Thr Leu Val Pro Val Thr Arg Asn Asp Ile Val Lys Ile Glu Gly
 225 230 235 240
 Ile Asp Ala Thr Gly Gly Asn Asn Gln Pro Asn Ile Pro Asp Ile Pro
 245 250 255
 Ala His Leu Trp Tyr Phe Gly Leu Ile Gly Thr Cys Leu
 260 265

<210> 2

<211> 481

<212> PRT

<213> SP722

<400> 2

Thr Asn Gly Thr Met Met Gln Tyr Phe Glu Trp His Leu Pro Asn Asp
 1 5 10 15
 Gly Asn His Trp Asn Arg Leu Arg Asp Asp Ala Ser Asn Leu Arg Asn
 20 25 30
 Arg Gly Ile Thr Ala Ile Trp Ile Pro Pro Ala Trp Lys Gly Thr Ser
 35 40 45
 Gln Asn Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr Asp Leu Gly Glu
 50 55 60
 Phe Asn Gln Lys Gly Thr Val Arg Thr Lys Tyr Gly Thr Arg Ser Gln
 65 70 75 80
 Leu Glu Ser Ala Ile His Ala Leu Lys Asn Asn Gly Val Gln Val Tyr
 85 90 95
 Gly Asp Val Val Met Asn His Lys Gly Gly Ala Asp Ala Thr Glu Asn
 100 105 110
 Val Leu Ala Val Glu Val Asn Pro Asn Asn Arg Asn Gln Glu Ile Ser
 115 120 125

Gly Asp Tyr Thr Ile Glu Ala Trp Thr Lys Phe Asp Phe Pro Gly Arg
 130 135 140
 Gly Asn Thr Tyr Ser Asp Phe Lys Trp Arg Trp Tyr His Phe Asp Gly
 145 150 155 160
 Val Asp Trp Asp Gln Ser Arg Gln Phe Gln Asn Arg Ile Tyr Lys Phe
 165 170 175
 Arg Gly Asp Gly Lys Ala Trp Asp Trp Glu Val Asp Ser Glu Asn Gly
 180 185 190
 Asn Tyr Asp Tyr Leu Met Tyr Ala Asp Val Asp Met Asp His Pro Glu
 195 200 205
 Val Val Asn Glu Leu Arg Arg Trp Gly Glu Trp Tyr Thr Asn Thr Leu
 210 215 220
 Asn Leu Asp Gly Phe Arg Ile Asp Ala Val Lys His Ile Lys Tyr Ser
 225 230 235 240
 Phe Thr Arg Asp Trp Leu Thr His Val Arg Asn Ala Thr Gly Lys Glu
 245 250 255
 Met Phe Ala Val Ala Glu Phe Trp Lys Asn Asp Leu Gly Ala Leu Glu
 260 265 270
 Asn Tyr Leu Asn Lys Thr Asn Trp Asn His Ser Val Phe Asp Val Pro
 275 280 285
 Leu His Tyr Asn Leu Tyr Asn Ala Ser Asn Ser Gly Gly Asn Tyr Asp
 290 295 300
 Met Ala Lys Leu Leu Asn Gly Thr Val Val Gln Lys His Pro Met His
 305 310 315 320
 Ala Val Thr Phe Val Asp Asn His Asp Ser Gln Pro Gly Glu Ser Leu
 325 330 335
 Glu Ser Phe Val Gln Glu Trp Phe Lys Pro Leu Ala Tyr Ala Leu Ile
 340 345 350
 Leu Thr Arg Glu Gln Gly Tyr Pro Ser Val Phe Tyr Gly Asp Tyr Tyr
 355 360 365
 Gly Ile Pro Thr His Ser Val Pro Ala Met Lys Ala Lys Ile Asp Pro
 370 375 380
 Ile Leu Glu Ala Arg Gln Asn Phe Ala Tyr Gly Thr Gln His Asp Tyr
 385 390 395 400
 Phe Asp His His Asn Ile Ile Gly Trp Thr Arg Glu Gly Asn Thr Thr
 405 410 415
 His Pro Asn Ser Gly Leu Ala Thr Ile Met Ser Asp Gly Pro Gly Gly
 420 425 430
 Glu Lys Trp Met Tyr Val Gly Gln Asn Lys Ala Gly Gln Val Trp His
 435 440 445

Asp Ile Thr Gly Asn Lys Pro Gly Thr Val Thr Ile Asn Ala Asp Gly
 450 455 460

Trp Ala Asn Phe Ser Val Asn Gly Gly Ser Val Ser Ile Trp Val Lys
 465 470 475 480

Arg

<210> 3

<211> 504

<212> PRT

<213> Coprinus cenerius

<400> 3

Gln Ile Val Asn Ser Val Asp Thr Met Thr Leu Thr Asn Ala Asn Val
 1 5 10 15

Ser Pro Asp Gly Phe Thr Arg Ala Gly Ile Leu Val Asn Gly Val His
 20 25 30

Gly Pro Leu Ile Arg Gly Gly Lys Asn Asp Asn Phe Glu Leu Asn Val
 35 40 45

Val Asn Asp Leu Asp Asn Pro Thr Met Leu Arg Pro Thr Ser Ile His
 50 55 60

Trp His Gly Leu Phe Gln Arg Gly Thr Asn Trp Ala Asn Gly Ala Asp
 65 70 75 80

Gly Val Asn Gln Cys Pro Ile Ser Pro Gly His Ala Phe Leu Tyr Lys
 85 90 95

Phe Thr Pro Ala Gly His Ala Gly Thr Phe Trp Tyr His Ser His Phe
 100 105 110

Gly Thr Gln Tyr Cys Asp Gly Leu Arg Gly Pro Met Val Ile Tyr Asp
 115 120 125

Asp Asn Asp Pro His Ala Ala Leu Tyr Asp Glu Asp Asp Glu Asn Thr
 130 135 140

Ile Ile Thr Leu Ala Asp Trp Tyr His Ile Pro Ala Pro Ser Ile Gln
 145 150 155 160

Gly Ala Ala Gln Pro Asp Ala Thr Leu Ile Asn Gly Lys Gly Arg Tyr
 165 170 175

Val Gly Gly Pro Ala Ala Glu Leu Ser Ile Val Asn Val Glu Gln Gly
 180 185 190

Lys Lys Tyr Arg Met Arg Leu Ile Ser Leu Ser Cys Asp Pro Asn Trp
 195 200 205

Gln Phe Ser Ile Asp Gly His Glu Leu Thr Ile Ile Glu Val Asp Gly
 210 215 220
 Asn Leu Thr Glu Pro His Thr Val Asp Arg Leu Gln Ile Phe Thr Gly
 225 230 235 240
 Gln Arg Tyr Ser Phe Val Leu Asp Ala Asn Gln Pro Val Asp Asn Tyr
 245 250 255
 Trp Ile Arg Ala Gln Pro Asn Lys Gly Arg Asn Gly Leu Ala Gly Thr
 260 265 270
 Phe Ala Asn Gly Val Asn Ser Ala Ile Leu Arg Tyr Ala Gly Ala Ala
 275 280 285
 Asn Ala Asp Pro Thr Thr Ser Ala Asn Pro Asn Pro Ala Gln Leu Asn
 290 295 300
 Glu Ala Asp Leu His Ala Leu Ile Asp Pro Ala Ala Pro Gly Ile Pro
 305 310 315 320
 Thr Pro Gly Ala Ala Asn Val Asn Leu Arg Phe Gln Leu Gly Phe Ser
 325 330 335
 Gly Gly Arg Phe Thr Ile Asn Gly Thr Ala Tyr Glu Ser Pro Ser Val
 340 345 350
 Pro Thr Leu Leu Gln Ile Met Ser Gly Ala Gln Ser Ala Asn Asp Leu
 355 360 365
 Leu Pro Ala Gly Ser Val Tyr Glu Leu Pro Arg Asn Gln Val Val Glu
 370 375 380
 Leu Val Val Pro Ala Gly Val Leu Gly Gly Pro His Pro Phe His Leu
 385 390 395 400
 His Gly His Ala Phe Ser Val Val Arg Ser Ala Gly Ser Ser Thr Tyr
 405 410 415
 Asn Phe Val Asn Pro Val Lys Arg Asp Val Val Ser Leu Gly Val Thr
 420 425 430
 Gly Asp Glu Val Thr Ile Arg Phe Val Thr Asp Asn Pro Gly Pro Trp
 435 440 445
 Phe Phe His Cys His Ile Glu Phe His Leu Met Asn Gly Leu Ala Ile
 450 455 460
 Val Phe Ala Glu Asp Met Ala Asn Thr Val Asp Ala Asn Asn Pro Pro
 465 470 475 480
 Val Glu Trp Ala Gln Leu Cys Glu Ile Tyr Asp Asp Leu Pro Pro Glu
 485 490 495
 Ala Thr Ser Ile Gln Thr Val Val
 500

<211> 213

<212> PRT

<213> Carezyme Core

<400> 4.

Ala Asp Gly Arg Ser Thr Arg Tyr Trp Asp Cys Cys Lys Pro Ser Cys
 1 5 10 15

Gly Trp Ala Lys Lys Ala Pro Val Asn Gln Pro Val Phe Ser Cys Asn
 20 25 30

Ala Asn Phe Gln Arg Ile Thr Asp Phe Asp Ala Lys Ser Gly Cys Glu
 35 40 45

Pro Gly Gly Val Ala Tyr Ser Cys Ala Asp Gln Thr Pro Trp Ala Val
 50 55 60

Asn Asp Asp Phe Ala Leu Gly Phe Ala Ala Thr Ser Ile Ala Gly Ser
 65 70 75 80

Asn Glu Ala Gly Trp Cys Cys Ala Cys Tyr Glu Leu Thr Phe Thr Ser
 85 90 95

Gly Pro Val Ala Gly Lys Lys Met Val Val Gln Ser Thr Ser Thr Gly
 100 105 110

Gly Asp Leu Gly Ser Asn His Phe Asp Leu Asn Ile Pro Gly Gly Gly
 115 120 125

Val Gly Ile Phe Asp Gly Cys Thr Pro Gln Phe Gly Gly Leu Pro Gly
 130 135 140

Gln Arg Tyr Gly Gly Ile Ser Ser Arg Asn Glu Cys Asp Arg Phe Pro
 145 150 155 160

Asp Ala Leu Lys Pro Gly Cys Tyr Trp Arg Phe Asp Trp Phe Lys Asn
 165 170 175

Ala Asp Asn Pro Ser Phe Ser Phe Arg Gln Val Gln Cys Pro Ala Glu
 180 185 190

Leu Val Ala Arg Thr Gly Cys Arg Arg Asn Asp Asp Gly Asn Phe Pro
 195 200 205

Ala Val Gln Ile Pro
 210

<210> 5

<211> 305

<212> PRT

<213> Carezyme full length (SwissProt accession number R15272)

<400> 5

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Met Arg Ser Ser Pro Leu Leu Pro Ser Ala Val Val Ala Ala Leu Pro
 1           5           10           15

Val Leu Ala Leu Ala Ala Asp Gly Arg Ser Thr Arg Tyr Trp Asp Cys
          20           25           30

Cys Lys Pro Ser Cys Gly Trp Ala Lys Lys Ala Pro Val Asn Gln Pro
          35           40           45

Val Phe Ser Cys Asn Ala Asn Phe Gln Arg Ile Thr Asp Phe Asp Ala
 50           55           60

Lys Ser Gly Cys Glu Pro Gly Gly Val Ala Tyr Ser Cys Ala Asp Gln
 65           70           75           80

Thr Pro Trp Ala Val Asn Asp Asp Phe Ala Leu Gly Phe Ala Ala Thr
          85           90           95

Ser Ile Ala Gly Ser Asn Glu Ala Gly Trp Cys Cys Ala Cys Tyr Glu
          100          105          110

Leu Thr Phe Thr Ser Gly Pro Val Ala Gly Lys Lys Met Val Val Gln
          115          120          125

Ser Thr Ser Thr Gly Gly Asp Leu Gly Ser Asn His Phe Asp Leu Asn
          130          135          140

Ile Pro Gly Gly Gly Val Gly Ile Phe Asp Gly Cys Thr Pro Gln Phe
          145          150          155          160

Gly Gly Leu Pro Gly Gln Arg Tyr Gly Gly Ile Ser Ser Arg Asn Glu
          165          170          175

Cys Asp Arg Phe Pro Asp Ala Leu Lys Pro Gly Cys Tyr Trp Arg Phe
          180          185          190

Asp Trp Phe Lys Asn Ala Asp Asn Pro Ser Phe Ser Phe Arg Gln Val
          195          200          205

Gln Cys Pro Ala Glu Leu Val Ala Arg Thr Gly Cys Arg Arg Asn Asp
          210          215          220

Asp Gly Asn Phe Pro Ala Val Gln Ile Pro Ser Ser Ser Thr Ser Ser
          225          230          235          240

Pro Val Asn Gln Pro Thr Ser Thr Ser Thr Thr Ser Thr Ser Thr Thr
          245          250          255

Ser Ser Pro Pro Val Gln Pro Thr Thr Pro Ser Gly Cys Thr Ala Glu
          260          265          270

Arg Trp Ala Gln Cys Gly Gly Asn Gly Trp Ser Gly Cys Thr Thr Cys
          275          280          285

Val Ala Gly Ser Thr Cys Thr Lys Ile Asn Asp Trp Tyr His Gln Cys
          290          295          300

```

Leu
305

<210> 6

<211> 159

<212> PRT

<213> Bet v1 sequence SwissProt accession number P15494)

<400> 6

Gly Val Phe Asn Tyr Glu Thr Glu Thr Thr Ser Val Ile Pro Ala Ala
1 5 10 15

Arg Leu Phe Lys Ala Phe Ile Leu Asp Gly Asp Asn Leu Phe Pro Lys
20 25 30

Val Ala Pro Gln Ala Ile Ser Ser Val Glu Asn Ile Glu Gly Asn Gly
35 40 45

Gly Pro Gly Thr Ile Lys Lys Ile Ser Phe Pro Glu Gly Phe Pro Phe
50 55 60

Lys Tyr Val Lys Asp Arg Val Asp Glu Val Asp His Thr Asn Phe Lys
65 70 75 80

Tyr Asn Tyr Ser Val Ile Glu Gly Gly Pro Ile Gly Asp Thr Leu Glu
85 90 95

Lys Ile Ser Asn Glu Ile Lys Ile Val Ala Thr Pro Asp Gly Gly Ser
100 105 110

Ile Leu Lys Ile Ser Asn Lys Tyr His Thr Lys Gly Asp His Glu Val
115 120 125

Lys Ala Glu Gln Val Lys Ala Ser Lys Glu Met Gly Glu Thr Leu Leu
130 135 140

Arg Ala Val Glu Ser Tyr Leu Leu Ala His Ser Asp Ala Tyr Asn
145 150 155

<210> 7

<211> 129

<212> PRT

<213> Der f2 (Dermatophagoides farinae allergen, pdb accession
number 1ahk.pdb)

<400> 7

Asp Gln Val Asp Val Lys Asp Cys Ala Asn Asn Glu Ile Lys Lys Val
1 5 10 15

```

Met Val Asp Gly Cys His Gly Ser Asp Pro Cys Ile Ile His Arg Gly
    20                      25                      30
Lys Pro Phe Thr Leu Glu Ala Leu Phe Asp Ala Asn Gln Asn Thr Lys
    35                      40                      45
Thr Ala Lys Ile Glu Ile Lys Ala Ser Leu Asp Gly Leu Glu Ile Asp
    50                      55                      60
Val Pro Gly Ile Asp Thr Asn Ala Cys His Phe Val Lys Cys Pro Leu
    65                      70                      75                      80
Val Lys Gly Gln Gln Tyr Asp Ile Lys Tyr Thr Trp Asn Val Pro Lys
    85                      90                      95
Ile Ala Pro Lys Ser Glu Asn Val Val Val Thr Val Lys Leu Ile Gly
    100                     105                     110
Asp Asn Gly Val Leu Ala Cys Ala Ile Ala Thr His Gly Lys Ile Arg
    115                     120                     125

```

Asp

<210> 8

<211> 129

<212> PRT

<213> Der p2 (Dermatophagoides pteronyssinus allergen, pdb accession number 1a9v.pdb)

<400> 8

```

Ser Gln Val Asp Val Lys Asp Cys Ala Asn His Glu Ile Lys Lys Val
1                      5                      10                      15
Leu Val Pro Gly Cys His Gly Ser Glu Pro Cys Ile Ile His Arg Gly
    20                      25                      30
Lys Pro Phe Gln Leu Glu Ala Val Phe Glu Ala Asn Gln Asn Thr Lys
    35                      40                      45
Thr Ala Lys Ile Glu Ile Lys Ala Ser Ile Asp Gly Leu Glu Val Asp
    50                      55                      60
Val Pro Gly Ile Asp Pro Asn Ala Cys His Tyr Met Lys Cys Pro Leu
    65                      70                      75                      80
Val Lys Gly Gln Gln Tyr Asp Ile Lys Tyr Thr Trp Asn Val Pro Lys
    85                      90                      95
Ile Ala Pro Lys Ser Glu Asn Val Val Val Thr Val Lys Val Met Gly
    100                     105                     110
Asp Asp Gly Val Leu Ala Cys Ala Ile Ala Thr His Ala Lys Ile Arg
    115                     120                     125

```

Asp

<210> 9

<211> 94

<212> PRT

<213> Phl p2 (allergen from pdb accession number 1whp.pdb)

<400> 9

```

Val Pro Lys Val Thr Phe Thr Val Glu Lys Gly Ser Asn Glu Lys His
1           5           10           15
Leu Ala Val Leu Val Lys Tyr Glu Gly Asp Thr Met Ala Glu Val Glu
          20           25           30
Leu Arg Glu His Gly Ser Asp Glu Trp Val Ala Met Thr Lys Gly Glu
          35           40           45
Gly Gly Val Trp Thr Phe Asp Ser Glu Glu Pro Leu Gln Gly Pro Phe
          50           55           60
Asn Phe Arg Phe Leu Thr Glu Lys Gly Met Lys Asn Val Phe Asp Asp
65           70           75           80
Val Val Pro Glu Lys Tyr Thr Ile Gly Ala Thr Tyr Ala Pro
          85           90

```

<210> 10

<211> 338

<212> PRT

<213> BPN' (Bacillus subtilis subtilisin from pdb accession number 1sib.pdb)

<400> 10

```

Ala Gln Ser Val Pro Tyr Gly Val Ser Gln Ile Lys Ala Pro Ala Leu
1           5           10           15
His Ser Gln Gly Tyr Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp
          20           25           30
Ser Gly Ile Asp Ser Ser His Pro Asp Leu Lys Val Ala Gly Gly Ala
          35           40           45
Ser Met Val Pro Ser Glu Thr Asn Pro Phe Gln Asp Asn Asn Ser His
          50           55           60
Gly Thr His Val Ala Gly Thr Val Ala Ala Leu Asn Asn Ser Ile Gly
65           70           75           80

```

[illegible]

<210> 11

<211> 268

<212> PRT

<213> Esperase (Bacillus subtilisin 147 from Bacillus lentus)

<400> 11

Gln Thr Val Pro Trp Gly Ile Ser Phe Ile Asn Thr Gln Gln Ala His
 1 5 10 15
 Asn Arg Gly Ile Phe Gly Asn Gly Ala Arg Val Ala Val Leu Asp Thr
 20 25 30
 Gly Ile Ala Ser His Pro Asp Leu Arg Ile Ala Gly Gly Ala Ser Phe
 35 40 45
 Ile Ser Ser Glu Pro Ser Tyr His Asp Asn Asn Gly His Gly Thr His
 50 55 60
 Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu Gly
 65 70 75 80
 Val Ala Pro Ser Ala Asp Leu Tyr Ala Val Lys Val Leu Asp Arg Asn
 85 90 95
 Gly Ser Gly Ser Leu Ala Ser Val Ala Gln Gly Ile Glu Trp Ala Ile
 100 105 110
 Asn Asn Asn Met His Ile Ile Asn Met Ser Leu Gly Ser Thr Ser Gly
 115 120 125
 Ser Ser Thr Leu Glu Leu Ala Val Asn Arg Ala Asn Asn Ala Gly Ile
 130 135 140
 Leu Leu Val Gly Ala Ala Gly Asn Thr Gly Arg Gln Gly Val Asn Tyr
 145 150 155 160
 Pro Ala Arg Tyr Ser Gly Val Met Ala Val Ala Ala Val Asp Gln Asn
 165 170 175
 Gly Gln Arg Ala Ser Phe Ser Thr Tyr Gly Pro Glu Ile Glu Ile Ser
 180 185 190
 Ala Pro Gly Val Asn Val Asn Ser Thr Tyr Thr Gly Asn Arg Tyr Val
 195 200 205
 Ser Leu Ser Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Val Ala
 210 215 220
 Ala Leu Val Lys Ser Arg Tyr Pro Ser Tyr Thr Asn Asn Gln Ile Arg
 225 230 235 240
 Gln Arg Ile Asn Gln Thr Ala Thr Tyr Leu Gly Ser Pro Ser Leu Tyr
 245 250 255
 Gly Asn Gly Leu Val His Ala Gly Arg Ala Thr Gln
 260 265

<210> 12

<211> 150

<212> PRT

<213> Bosd2

<400> 12

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Ile Asp Pro Ser Lys Ile Pro Gly Glu Trp Arg Ile Ile Tyr Ala Ala
1           5           10           15
Ala Asp Asn Lys Asp Lys Ile Val Glu Gly Gly Pro Leu Arg Asn Tyr
          20           25           30
Tyr Arg Arg Ile Glu Cys Ile Asn Asp Cys Glu Ser Leu Ser Ile Thr
          35           40           45
Phe Tyr Leu Lys Asp Gln Gly Thr Cys Leu Leu Leu Thr Glu Val Ala
          50           55           60
Lys Arg Gln Glu Gly Tyr Val Tyr Val Leu Glu Phe Tyr Gly Thr Asn
65           70           75           80
Thr Leu Glu Val Ile His Val Ser Glu Asn Met Leu Val Thr Tyr Val
          85           90           95
Glu Asn Tyr Asp Gly Glu Arg Ile Thr Lys Met Thr Glu Gly Leu Ala
          100          105          110
Lys Gly Thr Ser Phe Thr Pro Glu Glu Leu Glu Lys Tyr Gln Gln Leu
          115          120          125
Asn Ser Glu Arg Gly Val Pro Asn Glu Asn Ile Glu Asn Leu Ile Lys
          130          135          140

```

```

Thr Asp Asn Cys Pro Pro
145          150

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<210> 13

<211> 159

<212> PRT

<213> Equc1

<400> 13

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Val Ala Ile Arg Asn Phe Asp Ile Ser Lys Ile Ser Gly Glu Trp Tyr
1           5           10           15
Ser Ile Phe Leu Ala Ser Asp Val Lys Glu Lys Ile Glu Glu Asn Gly
          20           25           30
Ser Met Arg Val Phe Val Asp Val Ile Arg Ala Leu Asp Asn Ser Ser
          35           40           45
Leu Tyr Ala Glu Tyr Gln Thr Lys Val Asn Gly Glu Cys Thr Glu Phe
          50           55           60
Pro Met Val Phe Asp Lys Thr Glu Glu Asp Gly Val Tyr Ser Leu Asn
65           70           75           80

```

Tyr Asp Gly Tyr Asn Val Phe Arg Ile Ser Glu Phe Glu Asn Asp Glu
 85 90 95
 His Ile Ile Leu Tyr Leu Val Asn Phe Asp Lys Asp Arg Pro Phe Gln
 100 105 110
 Leu Phe Glu Phe Tyr Ala Arg Glu Pro Asp Val Ser Pro Glu Ile Lys
 115 120 125
 Glu Glu Phe Val Lys Ile Val Gln Lys Arg Gly Ile Val Lys Glu Asn
 130 135 140
 Ile Ile Asp Leu Thr Lys Ile Asp Arg Cys Phe Gln Leu Arg Gly
 145 150 155

<210> 14

<211> 269

<212> PRT

<213> Protease B

<400> 14

Ala Gln Thr Ile Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 5 10 15
 His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30
 Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45
 Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60
 His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80
 Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95
 Ser Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110
 Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125
 Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140
 Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser
 145 150 155 160
 Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190
 Met Ala Pro Gly Val Asn Ile Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205
 Ala Ser Asp Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220
 Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240
 Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255
 Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

<210> 15

<211> 129

<212> PRT

<213> Gald4

<400> 15

Lys Val Phe Gly Arg Cys Glu Leu Ala Ala Ala Met Lys Arg His Gly
 1 5 10 15
 Leu Asp Asn Tyr Arg Gly Tyr Ser Leu Gly Asn Trp Val Cys Ala Ala
 20 25 30
 Lys Phe Glu Ser Asn Phe Asn Thr Gln Ala Thr Asn Arg Asn Thr Asp
 35 40 45
 Gly Ser Thr Asp Tyr Gly Ile Leu Gln Ile Asn Ser Arg Trp Trp Cys
 50 55 60
 Asn Asp Gly Arg Thr Pro Gly Ser Arg Asn Leu Cys Asn Ile Pro Cys
 65 70 75 80
 Ser Ala Leu Leu Ser Ser Asp Ile Thr Ala Ser Val Asn Cys Ala Lys
 85 90 95
 Lys Ile Val Ser Asp Ala Asn Gly Met Asn Ala Trp Val Ala Trp Arg
 100 105 110
 Asn Arg Cys Lys Gly Thr Asp Val Gln Ala Trp Ile Arg Gly Cys Arg
 115 120 125
 Leu

<210> 16

<211> 260

<212> PRT

<213> Hevb8

<400> 16

Ser Trp Gln Thr Tyr Val Asp Asp His Leu Met Cys Asp Ile Asp Gly
 1 5 10 15
 His Arg Leu Thr Ala Ala Ala Ile Ile Gly His Asp Gly Ser Val Trp
 20 25 30
 Ala Gln Ser Ser Ser Phe Pro Gln Phe Lys Ser Asp Glu Val Ala Ala
 35 40 45
 Val Met Lys Asp Phe Asp Glu Pro Gly Ser Leu Ala Pro Thr Gly Leu
 50 55 60
 His Leu Gly Gly Thr Lys Tyr Met Val Ile Gln Gly Glu Pro Gly Ala
 65 70 75 80
 Val Ile Arg Gly Lys Lys Gly Ser Gly Gly Ile Thr Val Lys Arg Thr
 85 90 95
 Gly Gln Ala Leu Ile Ile Gly Ile Tyr Asp Glu Pro Leu Thr Pro Gly
 100 105 110
 Gln Cys Asn Met Ile Val Glu Arg Leu Gly Asp Tyr Leu Leu Asp Gln
 115 120 125
 Gly Leu Ser Trp Gln Thr Tyr Val Asp Asp His Leu Met Cys Asp Ile
 130 135 140
 Asp Gly His Arg Leu Thr Ala Ala Ala Ile Ile Gly His Asp Gly Ser
 145 150 155 160
 Val Trp Ala Gln Ser Ser Ser Phe Pro Gln Phe Lys Ser Asp Glu Val
 165 170 175
 Ala Ala Val Met Lys Asp Phe Asp Glu Pro Gly Ser Leu Ala Pro Thr
 180 185 190
 Gly Leu His Leu Gly Gly Thr Lys Tyr Met Val Ile Gln Gly Glu Pro
 195 200 205
 Gly Ala Val Ile Arg Gly Lys Lys Gly Ser Gly Gly Ile Thr Val Lys
 210 215 220
 Arg Thr Gly Gln Ala Leu Ile Ile Gly Ile Tyr Asp Glu Pro Leu Thr
 225 230 235 240
 Pro Gly Gln Cys Asn Met Ile Val Glu Arg Leu Gly Asp Tyr Leu Leu
 245 250 255
 Asp Gln Gly Leu
 260

<210> 17

<211> 125

<212> PRT

<213> Profilin1-AC

<400> 17

Ser Trp Gln Thr Tyr Val Asp Thr Asn Leu Val Gly Thr Gly Ala Val
 1 5 10 15
 Thr Gln Ala Ala Ile Leu Gly Leu Asp Gly Asn Thr Trp Ala Thr Ser
 20 25 30
 Ala Gly Phe Ala Val Thr Pro Ala Gln Gly Gln Thr Leu Ala Ser Ala
 35 40 45
 Phe Asn Asn Ala Asp Pro Ile Arg Ala Ser Gly Phe Asp Leu Ala Gly
 50 55 60
 Val His Tyr Val Thr Leu Arg Ala Asp Asp Arg Ser Ile Tyr Gly Lys
 65 70 75 80
 Lys Gly Ser Ala Gly Val Ile Thr Val Lys Thr Ser Lys Ser Ile Leu
 85 90 95
 Val Gly Val Tyr Asn Glu Lys Ile Gln Pro Gly Thr Ala Ala Asn Val
 100 105 110
 Val Glu Lys Leu Ala Asp Tyr Leu Ile Gly Gln Gly Phe
 115 120 125

<210> 18

<211> 130

<212> PRT

<213> Profilin1-AT

<400> 18

Ser Trp Gln Ser Tyr Val Asp Asp His Leu Met Cys Asp Val Glu Gly
 1 5 10 15
 Asn His Leu Thr Ala Ala Ala Ile Leu Gly Gln Asp Gly Ser Val Trp
 20 25 30
 Ala Gln Ser Ala Lys Phe Pro Gln Leu Lys Pro Gln Glu Ile Asp Gly
 35 40 45
 Ile Lys Lys Asp Phe Glu Glu Pro Gly Phe Leu Ala Pro Thr Gly Leu
 50 55 60
 Phe Leu Gly Gly Glu Lys Tyr Met Val Ile Gln Gly Glu Gln Gly Ala
 65 70 75 80

Val Ile Arg Gly Lys Lys Gly Pro Gly Gly Val Thr Ile Lys Lys Thr
85 90 95

Asn Gln Ala Leu Val Phe Gly Phe Tyr Asp Glu Pro Met Thr Gly Gly
100 105 110

Gln Cys Asn Leu Val Val Glu Arg Leu Gly Asp Tyr Leu Ile Glu Ser
115 120 125

Glu Leu
130

<210> 19

<211> 250

<212> PRT

<213> Profilin2-AC

<400> 19

Ser Trp Gln Thr Tyr Val Asp Thr Asn Leu Val Gly Thr Gly Ala Val
1 5 10 15

Thr Gln Ala Ala Ile Ile Gly His Asp Gly Asn Thr Trp Ala Thr Ser
20 25 30

Ala Gly Phe Ala Val Ser Pro Ala Asn Gly Ala Ala Leu Ala Asn Ala
35 40 45

Phe Lys Asp Ala Thr Ala Ile Arg Ser Asn Gly Phe Glu Leu Ala Gly
50 55 60

Thr Arg Tyr Val Thr Ile Arg Ala Asp Asp Arg Ser Val Tyr Gly Lys
65 70 75 80

Lys Gly Ser Ala Gly Val Ile Thr Val Lys Thr Ser Lys Ala Ile Leu
85 90 95

Ile Gly Val Tyr Asn Glu Lys Ile Gln Pro Gly Thr Ala Ala Asn Val
100 105 110

Val Glu Lys Leu Ala Asp Tyr Leu Ile Gly Gln Gly Phe Ser Trp Gln
115 120 125

Thr Tyr Val Asp Thr Asn Leu Val Gly Thr Gly Ala Val Thr Gln Ala
130 135 140

Ala Ile Ile Gly His Asp Gly Asn Thr Trp Ala Thr Ser Ala Gly Phe
145 150 155 160

Ala Val Ser Pro Ala Asn Gly Ala Ala Leu Ala Asn Ala Phe Lys Asp
165 170 175

Ala Thr Ala Ile Arg Ser Asn Gly Phe Glu Leu Ala Gly Thr Arg Tyr
180 185 190

Val Thr Ile Arg Ala Asp Asp Arg Ser Val Tyr Gly Lys Lys Gly Ser

195	200	205
Ala Gly Val Ile Thr Val Lys Thr Ser Lys Ala Ile Leu Ile Gly Val		
210	215	220
Tyr Asn Glu Lys Ile Gln Pro Gly Thr Ala Ala Asn Val Val Glu Lys		
225	230	235 240
Leu Ala Asp Tyr Leu Ile Gly Gln Gly Phe		
245	250	

<210> 20

<211> 123

<212> PRT

<213> Profilin-Birchpollen

<400> 20

Ser Trp Gln Thr Tyr Val Asp Glu His Leu Met Leu Ala Ala Ser Ala		
1	5	10 15
Ile Val Gly His Asp Gly Ser Val Trp Ala Gln Ser Ser Ser Phe Pro		
20	25	30
Gln Phe Lys Pro Gln Glu Ile Thr Gly Ile Met Lys Asp Phe Glu Glu		
35	40	45
Pro Gly His Leu Ala Pro Thr Gly Leu His Leu Gly Gly Ile Lys Tyr		
50	55	60
Met Val Ile Gln Gly Glu Ala Gly Ala Val Ile Arg Gly Lys Lys Gly		
65	70	75 80
Ser Gly Gly Ile Thr Ile Lys Lys Thr Gly Gln Ala Leu Val Phe Gly		
85	90	95
Ile Tyr Glu Glu Pro Val Thr Pro Gly Gln Cys Asn Met Val Val Glu		
100	105	110
Arg Leu Gly Asp Tyr Leu Ile Asp Gln Gly Leu		
115	120	

<210> 21

<211> 40

<212> PRT

<213> RagWeedpollen5

<400> 21

Asp Asp Gly Leu Cys Tyr Glu Gly Thr Asn Cys Gly Lys Val Gly Lys		
1	5	10 15

Tyr Cys Cys Ser Pro Ile Gly Lys Tyr Cys Val Cys Tyr Asp Ser Lys
 20 25 30

Ala Ile Cys Asn Lys Asn Cys Thr
 35 40

<210> 22

<211> 209

<212> PRT

<213> Vesv5

<400> 22

Ala Glu Ala Glu Phe Asn Asn Tyr Cys Lys Ile Lys Cys Leu Lys Gly
 1 5 10 15

Gly Val His Thr Ala Cys Lys Tyr Gly Ser Leu Lys Pro Asn Cys Gly
 20 25 30

Asn Lys Val Val Val Ser Tyr Gly Leu Thr Lys Gln Glu Lys Gln Asp
 35 40 45

Ile Leu Lys Glu His Asn Asp Phe Arg Gln Lys Ile Ala Arg Gly Leu
 50 55 60

Glu Thr Arg Gly Asn Pro Gly Pro Gln Pro Pro Ala Lys Asn Met Lys
 65 70 75 80

Asn Leu Val Trp Asn Asp Glu Leu Ala Tyr Val Ala Gln Val Trp Ala
 85 90 95

Asn Gln Cys Gln Tyr Gly His Asp Thr Cys Arg Asp Val Ala Lys Tyr
 100 105 110

Gln Val Gly Gln Asn Val Ala Leu Thr Gly Ser Thr Ala Ala Lys Tyr
 115 120 125

Asp Asp Pro Val Lys Leu Val Lys Met Trp Glu Asp Glu Val Lys Asp
 130 135 140

Tyr Asn Pro Lys Lys Lys Phe Ser Gly Asn Asp Phe Leu Lys Thr Gly
 145 150 155 160

His Tyr Thr Gln Met Val Trp Ala Asn Thr Lys Glu Val Gly Cys Gly
 165 170 175

Ser Ile Lys Tyr Ile Gln Glu Lys Trp His Lys His Tyr Leu Val Cys
 180 185 190

Asn Tyr Gly Pro Ser Gly Asn Phe Lys Asn Glu Glu Leu Tyr Gln Thr
 195 200 205

Lys

<210> 23

<211> 269

<212> PRT

<213> Protease B

<400> 23

Ala Gln Thr Ile Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15
 His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30
 Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45
 Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60
 His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80
 Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95
 Ser Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110
 Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125
 Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140
 Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser
 145 150 155 160
 Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175
 Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190
 Met Ala Pro Gly Val Asn Ile Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205
 Ala Ser Asp Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220
 Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240
 Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255
 Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg

260 265

<210> 24

<211> 269

<212> PRT

<213> Savinase

<400> 24

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95

Ser Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
 115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

<210> 25

<211> 274

<212> PRT

<213> Alcalase

<400> 25

Ala Gln Thr Val Pro Tyr Gly Ile Pro Leu Ile Lys Ala Asp Lys Val
 1 5 10 15

Gln Ala Gln Gly Phe Lys Gly Ala Asn Val Lys Val Ala Val Leu Asp
 20 25 30

Thr Gly Ile Gln Ala Ser His Pro Asp Leu Asn Val Val Gly Gly Ala
 35 40 45

Ser Phe Val Ala Gly Glu Ala Tyr Asn Thr Asp Gly Asn Gly His Gly
 50 55 60

Thr His Val Ala Gly Thr Val Ala Ala Leu Asp Asn Thr Thr Gly Val
 65 70 75 80

Leu Gly Val Ala Pro Ser Val Ser Leu Tyr Ala Val Lys Val Leu Asn
 85 90 95

Ser Ser Gly Ser Gly Ser Tyr Ser Gly Ile Val Ser Gly Ile Glu Trp
 100 105 110

Ala Thr Thr Asn Gly Met Asp Val Ile Asn Met Ser Leu Gly Gly Ala
 115 120 125

Ser Gly Ser Thr Ala Met Lys Gln Ala Val Asp Asn Ala Tyr Ala Arg
 130 135 140

Gly Val Val Val Val Ala Ala Gly Asn Ser Gly Ser Ser Gly Asn
 145 150 155 160

Thr Asn Thr Ile Gly Tyr Pro Ala Lys Tyr Asp Ser Val Ile Ala Val
 165 170 175

Gly Ala Val Asp Ser Asn Ser Asn Arg Ala Ser Phe Ser Ser Val Gly
 180 185 190

Ala Glu Leu Glu Val Met Ala Pro Gly Ala Gly Val Tyr Ser Thr Tyr
 195 200 205

Pro Thr Asn Thr Tyr Ala Thr Leu Asn Gly Thr Ser Met Ala Ser Pro
 210 215 220

His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn Leu
 225 230 235 240

Ser Ala Ser Gln Val Arg Asn Arg Leu Ser Ser Thr Ala Thr Tyr Leu
245 250 255

Gly Ser Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Glu Ala Ala
260 265 270

Ala Gln

<210> 26

<211> 269

<212> PRT

<213> Protease B

<400> 26

Ala Gln Thr Ile Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Ser Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser
145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
180 185 190

Met Ala Pro Gly Val Asn Ile Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
195 200 205

Ala Ser Asp Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala

210 215 220
 Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240
 Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255
 Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

 <210> 27

 <211> 269

 <212> PRT

 <213> Protease C

 <400> 27

 Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15
 His Asn Arg Gly Leu Thr Gly Ser Gly Val Arg Val Ala Val Leu Asp
 20 25 30
 Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
 35 40 45
 Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
 50 55 60
 His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
 65 70 75 80
 Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
 85 90 95
 Ser Gly Ser Gly Ser Tyr Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
 100 105 110
 Gly Asn Asn Gly Met His Val Ala Ser Leu Ser Leu Gly Ser Pro Ser
 115 120 125
 Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140
 Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser
 145 150 155 160
 Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175
 Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190
 Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Ala Arg
260 265

<210> 28

<211> 269

<212> PRT

<213> Protease D

<400> 28

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asp Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Ser Gly Ser Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser
145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
260 265

<210> 29

<211> 269

<212> PRT

<213> Protease E

<400> 29

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Ser Gly Gly Gly Ala Ile Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Asp Ser Ile Ser
145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile

180				185				190							
Val	Ala	Pro	Gly	Val	Asn	Val	Gln	Ser	Thr	Tyr	Pro	Gly	Ser	Thr	Tyr
195				200				205							
Ala	Ser	Leu	Asn	Gly	Thr	Ser	Met	Ala	Thr	Pro	His	Val	Ala	Gly	Ala
210				215				220							
Ala	Val	Leu	Val	Lys	His	Lys	Asn	Pro	Ser	Trp	Ser	Asn	Val	Arg	Ile
225				230				235				240			
Arg	Asp	His	Leu	Lys	Lys	Thr	Ala	Thr	Ser	Leu	Gly	Ser	Thr	Asn	Leu
245				250				255							
Tyr	Gly	Ser	Gly	Leu	Val	Asn	Ala	Glu	Ala	Ala	Thr	Arg			
260				265											

<210> 30
<211> 269
<212> PRT
<213> Protease A

<400> 30

Ala	Gln	Ser	Val	Pro	Trp	Gly	Ile	Ser	Arg	Val	Gln	Ala	Pro	Ala	Ala
1				5					10					15	
His	Asn	Arg	Gly	Leu	Thr	Gly	Ser	Gly	Val	Lys	Val	Ala	Val	Leu	Asp
			20					25					30		
Thr	Gly	Ile	Ser	Thr	His	Pro	Asp	Leu	Asn	Ile	Arg	Gly	Gly	Ala	Ser
		35					40					45			
Phe	Val	Pro	Gly	Glu	Pro	Ser	Thr	Gln	Asp	Gly	Asn	Gly	His	Gly	Thr
	50					55					60				
His	Val	Ala	Gly	Thr	Ile	Ala	Ala	Leu	Asn	Asn	Ser	Ile	Gly	Val	Leu
65					70					75					80
Gly	Val	Ala	Pro	Ser	Ala	Glu	Leu	Tyr	Ala	Val	Lys	Val	Leu	Gly	Ala
			85						90				95		
Ser	Gly	Ser	Gly	Ser	Val	Ser	Ser	Ile	Ala	Gln	Gly	Leu	Glu	Trp	Ala
			100					105					110		
Gly	Asn	Asn	Gly	Met	His	Val	Ala	Asn	Leu	Ser	Leu	Gly	Ser	Pro	Ser
		115					120					125			
Ala	Gly	Gly	Thr	Leu	Glu	Gln	Ala	Val	Asn	Ser	Ala	Thr	Ser	Arg	Gly
	130					135					140				
Val	Leu	Val	Val	Ala	Ala	Ser	Gly	Asn	Ser	Gly	Ala	Gly	Ser	Ile	Ser
145					150					155					160
Ala	Pro	Ala	Ser	Tyr	Ala	Asn	Ala	Met	Ala	Val	Gly	Ala	Thr	Asp	Gln
			165						170					175	

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Pro Gly Leu Asp Ile
180 185 190

Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
195 200 205

Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
225 230 235 240

Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
245 250 255

Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
260 265

<210> 31

<211> 269

<212> PRT

<213> Properase

<400> 31

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1 5 10 15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
65 70 75 80

Gly Val Ala Pro Asn Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Ser Gly Gly Gly Ser Asn Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
100 105 110

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
115 120 125

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
130 135 140

Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser
145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175
 Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190
 Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205
 Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220
 Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240
 Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255
 Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

<210> 32

<211> 270

<212> PRT

<213> Release

<400> 32

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
 1 5 10 15
 His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
 20 25 30
 Thr Gly Ile Asp Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala
 35 40 45
 Ser Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly
 50 55 60
 Thr His Val Ala Gly Thr Ile Ala Ala Leu Asp Asn Ser Ile Gly Val
 65 70 75 80
 Leu Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly
 85 90 95
 Ala Ser Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Glu Trp
 100 105 110
 Ala Gly Asn Asn Gly Met Asp Val Ala Asn Leu Ser Leu Gly Ser Pro
 115 120 125
 Ser Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg
 130 135 140
 Gly Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile

145 150 155 160
 Ser Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp
 165 170 175
 Gln Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Glu Leu Asp
 180 185 190
 Ile Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr
 195 200 205
 Tyr Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly
 210 215 220
 Ala Ala Ala Leu Val Leu Gln Lys Asn Pro Ser Trp Ser Asn Val Gln
 225 230 235 240
 Ile Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn
 245 250 255
 Leu Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265 270

<210> 33

<211> 280

<212> PRT

<213> PD498

<400> 33

Trp Ser Pro Asn Asp Pro Tyr Tyr Ser Ala Tyr Gln Tyr Gly Pro Gln
 1 5 10 15
 Asn Thr Ser Thr Pro Ala Ala Trp Asp Val Thr Arg Gly Ser Ser Thr
 20 25 30
 Gln Thr Val Ala Val Leu Asp Ser Gly Val Asp Tyr Asn His Pro Asp
 35 40 45
 Leu Ala Arg Lys Val Ile Lys Gly Tyr Asp Phe Ile Asp Arg Asp Asn
 50 55 60
 Asn Pro Met Asp Leu Asn Gly His Gly Thr His Val Ala Gly Thr Val
 65 70 75 80
 Ala Ala Asp Thr Asn Asn Gly Ile Gly Val Ala Gly Met Ala Pro Asp
 85 90 95
 Thr Lys Ile Leu Ala Val Arg Val Leu Asp Ala Asn Gly Ser Gly Ser
 100 105 110
 Leu Asp Ser Ile Ala Ser Gly Ile Arg Tyr Ala Ala Asp Gln Gly Ala
 115 120 125
 Lys Val Leu Asn Leu Ser Leu Gly Cys Glu Cys Asn Ser Thr Thr Leu
 130 135 140

Lys Ser Ala Val Asp Tyr Ala Trp Asn Lys Gly Ala Val Val Val Ala
145 150 155 160

Ala Ala Gly Asn Asp Asn Val Ser Arg Thr Phe Gln Pro Ala Ser Tyr
165 170 175

Pro Asn Ala Ile Ala Val Gly Ala Ile Asp Ser Asn Asp Arg Lys Ala
180 185 190

Ser Phe Ser Asn Tyr Gly Thr Trp Val Asp Val Thr Ala Pro Gly Val
195 200 205

Asn Ile Ala Ser Thr Val Pro Asn Asn Gly Tyr Ser Tyr Met Ser Gly
210 215 220

Thr Ser Met Ala Ser Pro His Val Ala Gly Leu Ala Ala Leu Leu Ala
225 230 235 240

Ser Gln Gly Lys Asn Asn Val Gln Ile Arg Gln Ala Ile Glu Gln Thr
245 250 255

Ala Asp Lys Ile Ser Gly Thr Gly Thr Asn Phe Lys Tyr Gly Lys Ile
260 265 270

Asn Ser Asn Lys Ala Val Arg Tyr
275 280

<210> 34

<211> 269

<212> PRT

<213> Sendai

<400> 34

Asn Gln Val Thr Pro Trp Gly Ile Thr Arg Val Gln Ala Pro Thr Ala
1 5 10 15

Trp Thr Arg Gly Tyr Thr Gly Thr Gly Val Arg Val Ala Val Leu Asp
20 25 30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Val Ser
35 40 45

Phe Val Pro Gly Glu Pro Ser Tyr Gln Asp Gly Asn Gly His Gly Thr
50 55 60

His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Val
65 70 75 80

Gly Val Ala Pro Asn Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
85 90 95

Asn Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Gln Trp Thr
100 105 110

Ala Gln Asn Asn Ile His Val Ala Asn Leu Ser Leu Gly Ser Pro Val
 115 120 125

Gly Ser Gln Thr Leu Glu Leu Ala Val Asn Gln Ala Thr Asn Ala Gly
 130 135 140

Val Leu Val Val Ala Ala Thr Gly Asn Asn Gly Ser Gly Thr Val Ser
 145 150 155 160

Tyr Pro Ala Arg Tyr Ala Asn Ala Leu Ala Val Gly Ala Thr Asp Gln
 165 170 175

Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Thr Gly Leu Asn Ile
 180 185 190

Val Ala Pro Gly Val Gly Ile Gln Ser Thr Tyr Pro Gly Asn Arg Tyr
 195 200 205

Ala Ser Leu Ser Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Val
 210 215 220

Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Thr Gln Ile
 225 230 235 240

Arg Gln His Leu Thr Ser Thr Ala Thr Ser Leu Gly Asn Ser Asn Gln
 245 250 255

Phe Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

<210> 35

<211> 268

<212> PRT

<213> YAB protease

<400> 35

Gln Thr Val Pro Trp Gly Ile Asn Arg Val Gln Ala Pro Ile Ala Gln
 1 5 10 15

Ser Arg Gly Phe Thr Gly Thr Gly Val Arg Val Ala Val Leu Asp Thr
 20 25 30

Gly Ile Ser Asn His Ala Asp Leu Arg Ile Arg Gly Gly Ala Ser Phe
 35 40 45

Val Pro Gly Glu Pro Asn Ile Ser Asp Gly Asn Gly His Gly Thr Gln
 50 55 60

Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu Gly
 65 70 75 80

Val Ala Pro Asn Val Asp Leu Tyr Gly Val Lys Val Leu Gly Ala Ser
 85 90 95

Gly Ser Gly Ser Ile Ser Gly Ile Ala Gln Gly Leu Gln Trp Ala Ala

100	105	110
Asn Asn Gly Met His Ile Ala	Asn Met Ser Leu Gly Ser Ser Ala Gly	
115	120	125
Ser Ala Thr Met Glu Gln Ala Val	Asn Gln Ala Thr Ala Ser Gly Val	
130	135	140
Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Asn Val Gly Phe		
145	150	155
Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln Asn		
165	170	175
Asn Asn Arg Ala Thr Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile Val		
180	185	190
Ala Pro Gly Val Gly Val Gln Ser Thr Val Pro Gly Asn Gly Tyr Ala		
195	200	205
Ser Phe Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Val Ala		
210	215	220
Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile Arg		
225	230	235
Asn His Leu Lys Asn Thr Ala Thr Asn Leu Gly Asn Thr Thr Gln Phe		
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Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg		
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<211> 471

<212> PRT

<213> AMG

<400> 36

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Ala Ile Leu Asn Asn Ile Gly Ala Asp Gly Ala Trp Val Ser Gly Ala	
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Asp Ser Gly Ile Val Val Ala Ser Pro Ser Thr Asp Asn Pro Asp Tyr	
35	45
Phe Tyr Thr Trp Thr Arg Asp Ser Gly Leu Val Leu Lys Thr Leu Val	
50	60
Asp Leu Phe Arg Asn Gly Asp Thr Ser Leu Leu Ser Thr Ile Glu Asn	
65	80
Tyr Ile Ser Ala Gln Ala Ile Val Gln Gly Ile Ser Asn Pro Ser Gly	
85	95

Asp Leu Ser Ser Gly Ala Gly Leu Gly Glu Pro Lys Phe Asn Val Asp
 100 105 110
 Glu Thr Ala Tyr Thr Gly Ser Trp Gly Arg Pro Gln Arg Asp Gly Pro
 115 120 125
 Ala Leu Arg Ala Thr Ala Met Ile Gly Phe Gly Gln Trp Leu Leu Asp
 130 135 140
 Asn Gly Tyr Thr Ser Thr Ala Thr Asp Ile Val Trp Pro Leu Val Arg
 145 150 155 160
 Asn Asp Leu Ser Tyr Val Ala Gln Tyr Trp Asn Gln Thr Gly Tyr Asp
 165 170 175
 Leu Trp Glu Glu Val Asn Gly Ser Ser Phe Phe Thr Ile Ala Val Gln
 180 185 190
 His Arg Ala Leu Val Glu Gly Ser Ala Phe Ala Thr Ala Val Gly Ser
 195 200 205
 Ser Cys Ser Trp Cys Asp Ser Gln Ala Pro Glu Ile Leu Cys Tyr Leu
 210 215 220
 Gln Ser Phe Trp Thr Gly Ser Phe Ile Leu Ala Asn Phe Asp Ser Ser
 225 230 235 240
 Arg Ser Gly Lys Asp Ala Asn Thr Leu Leu Gly Ser Ile His Thr Phe
 245 250 255
 Asp Pro Glu Ala Ala Cys Asp Asp Ser Thr Phe Gln Pro Cys Ser Pro
 260 265 270
 Arg Ala Leu Ala Asn His Lys Glu Val Val Asp Ser Phe Arg Ser Ile
 275 280 285
 Tyr Thr Leu Asn Asp Gly Leu Ser Asp Ser Glu Ala Val Ala Val Gly
 290 295 300
 Arg Tyr Pro Glu Asp Thr Tyr Tyr Asn Gly Asn Pro Trp Phe Leu Cys
 305 310 315 320
 Thr Leu Ala Ala Ala Glu Gln Leu Tyr Asp Ala Leu Tyr Gln Trp Asp
 325 330 335
 Lys Gln Gly Ser Leu Glu Val Thr Asp Val Ser Leu Asp Phe Phe Lys
 340 345 350
 Ala Leu Tyr Ser Asp Ala Ala Thr Gly Thr Tyr Ser Ser Ser Ser
 355 360 365
 Thr Tyr Ser Ser Ile Val Asp Ala Val Lys Thr Phe Ala Asp Gly Phe
 370 375 380
 Val Ser Ile Val Glu Thr His Ala Ala Ser Asn Gly Ser Met Ser Glu
 385 390 395 400
 Gln Tyr Asp Lys Ser Asp Gly Glu Gln Leu Ser Ala Arg Asp Leu Thr
 405 410 415

Trp Ser Tyr Ala Ala Leu Leu Thr Ala Asn Asn Arg Arg Asn Ser Val
 420 425 430

Val Pro Ala Ser Trp Gly Glu Thr Ser Ala Ser Ser Val Pro Gly Thr
 435 440 445

Cys Ala Ala Thr Ser Ala Ile Gly Thr Tyr Ser Ser Val Thr Val Thr
 450 455 460

Ser Trp Pro Ser Ile Val Ala
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<213> AA560

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Thr Asn Gly Thr Met Met Gln Tyr Phe Glu Trp Tyr Leu Pro Asn Asp
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Lys Gly Ile Ser Ala Val Trp Ile Pro Pro Ala Trp Lys Gly Ala Ser
 35 40 45

Gln Asn Asp Val Gly Tyr Gly Ala Tyr Asp Leu Tyr Asp Leu Gly Glu
 50 55 60

Phe Asn Gln Lys Gly Thr Ile Arg Thr Lys Tyr Gly Thr Arg Asn Gln
 65 70 75 80

Leu Gln Ala Ala Val Asn Ala Leu Lys Ser Asn Gly Ile Gln Val Tyr
 85 90 95

Gly Asp Val Val Met Asn His Lys Gly Gly Ala Asp Ala Thr Glu Met
 100 105 110

Val Arg Ala Val Glu Val Asn Pro Asn Asn Arg Asn Gln Glu Val Ser
 115 120 125

Gly Glu Tyr Thr Ile Glu Ala Trp Thr Lys Phe Asp Phe Pro Gly Arg
 130 135 140

Gly Asn Thr His Ser Asn Phe Lys Trp Arg Trp Tyr His Phe Asp Gly
 145 150 155 160

Val Asp Trp Asp Gln Ser Arg Lys Leu Asn Asn Arg Ile Tyr Lys Phe
 165 170 175

Arg Gly Asp Gly Lys Gly Trp Asp Trp Glu Val Asp Thr Glu Asn Gly
 180 185 190

Asn Tyr Asp Tyr Leu Met Tyr Ala Asp Ile Asp Met Asp His Pro Glu

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Val	Val	Asn	Glu	Leu	Arg	Asn	Trp	Gly	Val	Trp	Tyr	Thr	Asn	Thr	Leu
210						215					220				
Gly	Leu	Asp	Gly	Phe	Arg	Ile	Asp	Ala	Val	Lys	His	Ile	Lys	Tyr	Ser
225						230					235				240
Phe	Thr	Arg	Asp	Trp	Ile	Asn	His	Val	Arg	Ser	Ala	Thr	Gly	Lys	Asn
				245					250					255	
Met	Phe	Ala	Val	Ala	Glu	Phe	Trp	Lys	Asn	Asp	Leu	Gly	Ala	Ile	Glu
			260					265						270	
Asn	Tyr	Leu	Asn	Lys	Thr	Asn	Trp	Asn	His	Ser	Val	Phe	Asp	Val	Pro
		275					280					285			
Leu	His	Tyr	Asn	Leu	Tyr	Asn	Ala	Ser	Lys	Ser	Gly	Gly	Asn	Tyr	Asp
	290					295					300				
Met	Arg	Gln	Ile	Phe	Asn	Gly	Thr	Val	Val	Gln	Arg	His	Pro	Met	His
305						310					315				320
Ala	Val	Thr	Phe	Val	Asp	Asn	His	Asp	Ser	Gln	Pro	Glu	Glu	Ala	Leu
				325					330					335	
Glu	Ser	Phe	Val	Glu	Glu	Trp	Phe	Lys	Pro	Leu	Ala	Tyr	Ala	Leu	Thr
			340					345					350		
Leu	Thr	Arg	Glu	Gln	Gly	Tyr	Pro	Ser	Val	Phe	Tyr	Gly	Asp	Tyr	Tyr
		355					360					365			
Gly	Ile	Pro	Thr	His	Gly	Val	Pro	Ala	Met	Lys	Ser	Lys	Ile	Asp	Pro
	370					375					380				
Ile	Leu	Glu	Ala	Arg	Gln	Lys	Tyr	Ala	Tyr	Gly	Arg	Gln	Asn	Asp	Tyr
385						390					395				400
Leu	Asp	His	His	Asn	Ile	Ile	Gly	Trp	Thr	Arg	Glu	Gly	Asn	Thr	Ala
				405					410					415	
His	Pro	Asn	Ser	Gly	Leu	Ala	Thr	Ile	Met	Ser	Asp	Gly	Ala	Gly	Gly
			420				425						430		
Asn	Lys	Trp	Met	Phe	Val	Gly	Arg	Asn	Lys	Ala	Gly	Gln	Val	Trp	Thr
		435					440					445			
Asp	Ile	Thr	Gly	Asn	Arg	Ala	Gly	Thr	Val	Thr	Ile	Asn	Ala	Asp	Gly
	450					455					460				
Trp	Gly	Asn	Phe	Ser	Val	Asn	Gly	Gly	Ser	Val	Ser	Ile	Trp	Val	Asn
465						470					475				480

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